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Towards Democratization of Nucleic Acid Detection

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Thèse de doctorat de Sciences du vivant appliquées, biotechnologie et ingénierie des biosystèmes moléculaires

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"The greatest scientific discovery was the discovery of ignorance. Once humans realized how little they knew about the world, they suddenly had very good reason to seek new knowledge, which opened up the scientific road to progress."

"In the early twenty-first century the train of progress is again pulling out of the station -- and this will probably be the last train ever to leave the station called Homo sapiens. Those who miss this train will never get a second chance. In order to get a seat on it you need to understand twenty-first century technology, and in particular the powers of biotechnology and computer algorithms."

Yuval Noah Harari, Homo Deus: A brief history of tomorrow

"The DNA base pair will join the atom, the bit and the joule in the pantheon of elementary units from which new things are made. But DNA is not like the others. Everything we eat, everyone we love, sickness and health, the entire living world is written in DNA which therefore belongs in the public domain."

Jake Wintermute, The Free DNA Declaration

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Abstract

The aim of this project was to understand how to democratize nucleic acid detection and how to harness it for citizen science and education. Allowing anybody anywhere to do and understand genetic detection, simply rapidly and affordably and to demystify, empower, educate, and inspire

This interdisciplinary work both used and developed novel tools at the intersection of Molecular Biology, Citizen/Open, and Learning Sciences. We tested new high throughput, low volume, and multi-parameter techniques to develop and optimize fluorescent isothermal nucleic acid amplification assays which are not only rapid, sensitive, and specific but also **robust**. We created a 5 minutes DNA extraction protocol that only needs water. Detection is done utilizing our ultra-affordable (<2$) easy to build open-hardware fluorescence detector.

Making finding a specific fragment of DNA/RNA more accessible by reducing the cost of the reactions and instrumentation by at least an order of magnitude and halving the length of the experiments as compared to traditional PCR. Additionally simplifying it, such that even the untrained public (from the ages of 5 to 85) can successfully detect and see a gene with their own eyes. The hour of incubation time allows for deeper discussion, learning and debate. The first use case is detection of GMO's in food and feed.

This has all been packaged into a modular open Workshop/Lab that has been adapted to different audiences. The workshop has been done over 25 times in 5 countries (France, UK, Switzerland, USA, Spain), by more than 400 people, half of which were K-14 students in the Paris region, and the other half on diverse groups such as researchers, biohackers/makers, and the general public. Pre/post workshop questionnaires have shown significantly improved **understanding, empowerment and motivation** by the participants.

As Proof of the generality of this approach during the COVID pandemic these methodologies and the lessons learned have been applied to the detection of SARS-CoV-2 in an open and collaborative way with partners around the globe. Particularly focusing on solutions for low resource settings, which might lack access to infrastructure and robust cold chains.

We believe this is an important step towards making nucleic acids accessible to a wider audience, in an open, hands on, learning by doing way. This powerful methodology could be used for a variety of other targets such as interrogating the food we eat or searching for endangered, invasive or pathogenic species.

Résumé

L'objectif de ce projet était de comprendre comment démocratiser la détection des acides nucléiques et comment l'exploiter pour la science et l'éducation des citoyens. Permettre à quiconque, où à n'importe quel endroit, de faire et de comprendre la détection génétique, simplement, rapidement et à moindre coût, et de démystifier, habiliter, éduquer et inspirer.

Ce travail interdisciplinaire a à la fois utilisé et développé de nouveaux outils à l'intersection de la biologie moléculaire, des sciences citoyennes, ouverte et de l'apprentissage. Nous avons testé de nouvelles techniques à haut débit, à faible volume et multiparamétriques, pour développer et optimiser des tests d'amplification d'acides nucléiques isothermes fluorescents, qui sont non seulement rapides, sensibles et spécifiques, mais aussi robustes. Nous avons créé un protocole d'extraction d'ADN en 5 minutes qui ne nécessite que de l'eau. La détection se fait à l'aide de notre détecteur de fluorescence ultra abordable (<2$), facile à construire et ouvert.

Rendre la recherche d'un fragment spécifique d'ADN/ARN plus accessible en réduisant le coût des réactions et de l'instrumentation d'au moins un ordre de grandeur et en réduisant de moitié la durée des expériences par rapport à la PCR traditionnelle. En outre, elle est simplifiée, de sorte que même le public non formé (de 5 à 85 ans) peut détecter et voir un gène de ses propres yeux. L'heure d'incubation permet une discussion, un apprentissage et un débat plus approfondis. Le premier cas d'utilisation est la détection des OGM dans les denrées alimentaires et les plantes.

Tout cela a été regroupé dans un atelier/laboratoire ouvert et modulaire qui a été adapté à différents publics. L'atelier a été organisé plus de 25 fois dans 5 pays (France, Royaume-Uni, Suisse, États-Unis, Espagne), par plus de 400 personnes, dont la moitié étaient des élèves allant du college à la premiere annee de licence en région parisienne, et l'autre moitié des groupes divers tels que des chercheurs, des biohackers et des makers, et le grand public. Les questionnaires pré/post atelier ont montré une amélioration significative de la compréhension, de l'empowerment et de la motivation des participants.

Preuve de la généralité de cette approche pendant la pandémie de COVID, ces méthodologies et les leçons apprises ont été appliquées à la détection du SRAS-CoV-2 de manière ouverte et en collaboration avec des partenaires du monde entier. L'accent a été mis en particulier sur les solutions pour les environnements à faibles ressources, qui pourraient ne pas avoir accès aux infrastructures et à des chaînes du froid solides.

Nous pensons que c'est une étape importante pour rendre les acides nucléiques accessibles à un public plus large, de manière ouverte et participative, en apprenant par la pratique. Cette puissante méthodologie pourrait être utilisée pour toute une série d'autres cibles telles que l'interrogation de la nourriture que nous mangeons ou la recherche d'espèces menacées, envahissantes ou pathogènes.

Résumé détaillé

Ici, je présente l'utilisation et le développement des techniques d'amplification isotherme afin d'intéresser les étudiants et le public à la biotechnologie et à certaines de ses questions centrales. Il en résulte un kit/atelier éducatif stable, facile à utiliser, peu coûteux et efficace pour détecter les OGM dans les denrées alimentaires ou les aliments pour animaux. Après un bref historique de l'ADN, j'aborderai les techniques d'amplification isotherme comme alternative à la PCR, en particulier le LAMP (Loop mediated isothermal amplification) et la manière dont j'ai amélioré, optimisé et adapté les méthodes LAMP existantes pour la détection des OGM, à l'usage des néophytes (chapitre 2). Ensuite, j'aborderai certains des obstacles techniques qui empêchent de sortir ces réactions du laboratoire et de les transposer dans la "nature". Il s'agit notamment de l'extraction des acides nucléiques, de l'accès aux réactifs (wetware) et aux outils, du chauffage isotherme stable, ainsi que de la détection de l'amplification. Je montre comment nous avons surmonté tous ces obstacles, tout en réduisant le prix de chaque étape d'au moins un ordre de grandeur. Tout cela a été regroupé dans un atelier modulaire destiné aux néophytes et aux étudiants, qui a été réitéré et amélioré (chapitre 3). J'aborde ensuite les activités d'apprentissage pratique en général, et en biologie moléculaire en particulier, dans un cadre tant formel (école) qu'informel. Je montre comment nous avons mis en œuvre l'atelier et la méthodologie développés dans divers contextes et leur efficacité pour améliorer la compréhension, l'intérêt, le niveau de compétence et l'autonomisation (chapitre 4). Enfin, je conclus en abordant ces travaux dans un cadre plus large et je donne des pistes de travail et de recherche pour l'avenir (chapitre 5).

Bien que la détection des OGM par amplification en boucle ait été démontrée il y a 15 ans, elle n'a pas encore été largement adoptée. En le rendant plus facile à utiliser, abordable et robuste, nous avons pu utiliser ce test avec des centaines de néophytes dans le monde entier, comme un outil scientifique éducatif/citoyen. J'ai d'abord adapté les séries d'amorces et les réactions LAMP publiées pour les utiliser avec une enzyme WarmStart qui permet de mettre en place la réaction à température ambiante, ce qui évite d'avoir recours à la glace. Les réactions ont également été réduites pour des raisons de coût. En outre, les amorces ont été

adaptées de manière à ce que les réactions soient plus résistantes au problème fréquent des faux positifs, et que l'amplification puisse être détectée facilement par fluorescence avec une optique simple, en adoptant la nouvelle méthode QUASR proposée. Cela pourrait également permettre le multiplexage à l'avenir. En utilisant de nouvelles méthodes à haut débit, j'ai pu optimiser les réactions non seulement pour leur sensibilité et leur spécificité, comme c'est habituellement le cas, mais aussi pour leur caractère économique et leur robustesse face aux erreurs de pipetage.

La promesse des essais LAMP a toujours été qu'ils sont plus simples, plus rapides et nécessitent moins de formation et d'instrumentation. Bien que cela ait été réalisé dans de nombreux cas auparavant, la plupart des solutions trouvées jusqu'à présent ne sont pas dimensionnées de manière à ce que des dizaines ou des centaines de tests puissent être effectués en même temps, en particulier par des néophytes. J'ai amélioré les tests LAMP existants en les rendant plus robustes, résistants aux faux-positifs, détectables par fluorescence et stables pendant au moins deux mois à température ambiante par lyophilisation. Ce test a été couplé à un simple protocole de préparation de l'ADN en 5 minutes qui ne nécessite qu'un tube de 1,5 ml, un pilon et de l'eau. J'ai également mis au point du matériel à code source ouvert et des "hacks". Notre détecteur extrêmement économique, facile à construire et à réparer, permet une lecture simple et instantanée des résultats à l'œil nu ou par caméra. Ce matériel open source pourrait également être utilisé pour d'autres tests fluorescents, en particulier dans des environnements à faibles ressources. Il est important de noter que j'ai également pu réduire le prix des réactions et du matériel nécessaire d'au moins un ordre de grandeur par rapport à ce qu'ils coûtent habituellement au laboratoire, de sorte que cela peut être fait n'importe où par n'importe qui.

Tout cela a été regroupé dans un atelier modulaire ouvert qui peut facilement être adapté à différents publics. La construction des détecteurs à partir de pièces détachées, même avec des personnes qui n'ont jamais soudé auparavant, prend moins d'une heure. L'expérience de biologie prend également moins d'une heure, même avec des personnes n'ayant jamais utilisé de micropipette. L'heure d'incubation peut être utilisée pour discuter de questions plus profondes, ou les résultats peuvent être analysés à une date différente car ils sont stables pendant des semaines. Tout ce dont vous avez besoin pour construire le matériel et réaliser

l'expérience biologique pour 20 participants tient facilement dans un sac de transport de la taille d'un sac. Tous les différents aspects de ce travail sont open source et des guides détaillés sont disponibles en ligne de sorte que chacun peut les recréer lui-même, certains l'ont déjà fait en s'adressant au public et aux étudiants. J'ai volontairement essayé de rendre l'expérience et tous les autres aspects aussi faciles et ouverts que possible afin de permettre au plus grand nombre de personnes de participer, d'apprendre ou de la développer pour leur propre usage. Ce travail, ainsi que les premiers succès que nous avons obtenus en laboratoire avec divers groupes, nous ont donné la confiance nécessaire pour transposer l'atelier développé dans le monde réel.

Mes résultats indiquent qu'il y a au moins une place pour ce genre d'intervention, ne serait-ce que pour les réactions enthousiastes de tant de participants divers, de tous âges (12-75 ans) et de tous horizons, aux affirmations suivantes : j'ai découvert qu'il y a beaucoup de ressources à ma disposition. J'ai appris que je pouvais participer à la recherche scientifique. Et j'ai été intéressé par ce que j'ai fait et appris. En outre, il semble que tous les objectifs d'un laboratoire de sciences pratiques aient été atteints, dans un domaine difficile et peu étudié mais extrêmement pertinent, sans qu'il soit nécessaire de recourir à des appareils coûteux ou complexes. Nous avons démontré les points suivants :

- Amélioration de la maîtrise de plusieurs sujets.
- Développement du raisonnement scientifique, comprehension de la complexité et de l'ambiguïté du travail empirique et de la nature de la science.
- Développement des compétences pratiques telles que l'utilisation de micropipettes, la soudure et la construction de ses propres outils, ainsi que la conduite d'une expérience scientifique et la réflexion sur un sujet socio-scientifique avec un œil critique.
- Développement de l'intérêt pour les sciences et de l'intérêt pour l'apprentissage des sciences, en particulier pour les sujets qu'ils connaissaient peu auparavant et pas seulement de manière passive, mais aussi en étant prêt à investir activement du temps dans ces sujets.
- Et enfin, développement des capacités de travail en équipe, comme on a pu le constater lorsque les participants ont dû tenir soit des tubes à essai, soit un

circuit imprimé pendant que leurs coéquipiers essayaient de pipeter ou de souder de petits volumes ou des composants avec soin et précision.

Dans un cadre formel, les enseignants ont été particulièrement satisfaits des aspects pratiques ainsi que de l'adéquation au niveau de la classe et au programme d'études, et ont souligné les aspects actifs et interdisciplinaires qui ont facilité "...le décloisonnement des connaissances-compétences, contrairement au cloisonnement des disciplines imposé par le système éducatif national...(qui est) nécessaire à la compréhension de nombreux mécanismes complexes".

Ce travail multidisciplinaire et interdisciplinaire combinant et intégrant des outils moléculaires, numériques, physiques, éducatifs et scientifiques de manière innovante avait deux objectifs principaux : 1) abaisser la barre d'entrée, permettant à quiconque, où qu'il soit, de détecter un fragment d'ADN spécifique et 2) démystifier, responsabiliser, éduquer et inspirer de multiples publics, en leur permettant de rendre l'ADN plus concret, en le voyant de leurs propres yeux, et de fournir des outils qui leur permettent de renforcer leur confiance, leurs compétences et leur capacité d'action de leurs propres mains.

En se concentrant sur des méthodologies isothermes robustes, il est possible d'éviter le recours à des machines précises et coûteuses, à un personnel hautement qualifié et à des laboratoires spécialisés, en sortant ces outils de leur tour d'ivoire et en les rendant disponibles et faciles à utiliser, en particulier dans les régions où les ressources sont faibles. Ces nouveaux outils et astuces, partagés ouvertement et de manière accessible, pourraient permettre la détection de l'ADN dans une école, une cuisine, un bureau, un bidonville ou dans les champs. Permettant à quiconque un point d'entrée pour s'engager de manière significative avec l'importance en forte croissante des biotechnologies dans le monde.

Dans un monde où les lacunes en matière d'éducation ne cessent de se creuser, il est essentiel de familiariser une population toujours plus nombreuse d'apprenants avec les sciences de la vie modernes, en mettant l'accent sur la conception expérimentale, l'analyse des données, la pensée critique et le caractère ludique. J'espère que ce module et la méthodologie développée au cours de mon doctorat ne seront qu'une brique parmi d'autres.

Enfin, cette même méthodologie a été utilisée pour aider à lutter contre la pandémie de COVID : la surveillance pour identifier les personnes infectées par le CoV-2 du

SRAS avant une transmission à grande échelle est essentielle pour mettre fin à la pandémie de Covid-19, même si les vaccinations ont déjà commencé. Nous décrivons ici le Corona Detective, un test sensible et rapide basé sur l'AMPlification isotherme à médiation par boucle pour détecter le virus, qui pourrait être appliqué partout à faible coût. La méthode utilise essentiellement des réactifs lyophilisés, faciles à expédier sans dépendance à la chaîne du froid. Elle comprend une ADN polymérase capable de déplacer un brin à une seule température et des ensembles d'amorces pour détecter le gène N viral et un ARN cellulaire de contrôle, tous deux avec des lectures de fluorescence quenchées spécifiques à la séquence. Corona Detective peut être utilisé en bandes de 8 tubes pour être lu avec un simple détecteur de fluorescence à conception open source. D'autres méthodes permettant d'utiliser et de produire le Corona Detective localement sous différents formats sont possibles, et déjà ouvertement partagées. La spécificité de la détection est assurée par l'inclusion de réactions de témoins positifs et négatifs à exécuter en parallèle avec les réactions de diagnostic. Un protocole utilisateur simple, incluant la préparation des échantillons, et un pipeline bioinformatique pour garantir que les souches virales seront toujours détectables avec le jeu d'amorces SARS-CoV-2 choisi, complètent la méthode. Grâce à la production et à la distribution rapides de ces réactions, assez peu coûteuses à l'échelle, nous prévoyons, en association avec des campagnes de vaccination, des tests de surveillance quotidiens ou hebdomadaires sur de vastes populations, sans attendre l'apparition de symptômes pour les tester, afin de maîtriser enfin cette pandémie.

Titre de la thèse: Vers la démocratisation de la détection des acides nucléiques

Mots-clés: Education, ADN , OGM, Open-Source, LAMP, SARS-CoV-2, COVID-19

Keywords: Education, DNA, GMO, Open-Source, LAMP, SARS-CoV-2, COVID-19

Chapter 1:

Background and context

1.1 Background

Some personal background on how this thesis came about:
After completing a triple major bachelor's degree from TAU in General Humanities (philosophy, psychology, history, social justice, cognition...), Physics, and Biology, I went directly on to do an interdisciplinary masters at the Weizmann institute of Science. And although the Science and people there are of the highest quality, and although I was accepted to fully funded PhD programs both at the Weizmann and at Colombia University, at age 30 I felt like something was missing. I felt that a serious look at the social aspects of the science being done was missing. I turned them down, as the timing was off and I felt I wanted to create a bigger impact on the world.

I spent the next year trying to open an accelerator/BioHackerSpace in Israel with a partner, a place where people could come and do/make biology outside of an academic or corporate setting, this was difficult, without experience or credibility, but was going well and gaining traction. At the same time the news was filled with another pandemic, the Zika virus spreading rapidly through Brazil. As we were familiar with isothermal amplification based techniques for nucleic acid detection, and knew the infrastructure in Brazil was very limited, I rapidly put together a scientific proposal, which quickly gained interest, and next thing you know I found myself in northeastern Brazil, in the heart of the pandemic, presenting the original idea at an international conference:

We proposed to develop, test and deploy an, easy to use, rapid, reliable and economical RT-LAMP based diagnostic kit for ZIKV and other arboviruses, without the need of expensive, complex equipment or highly trained laboratory personnel,

which is suitable for low income and rural settings. This will be coupled to new and emerging open platforms for instantaneous data collection and dissemination. Empowering communities to create, share and access their data, exposing them to science, creating genetic literacy, and public health education, while facilitating accurate evidence-based responses by health organizations and development of better epidemiological models (**Fig 1.1.1**).

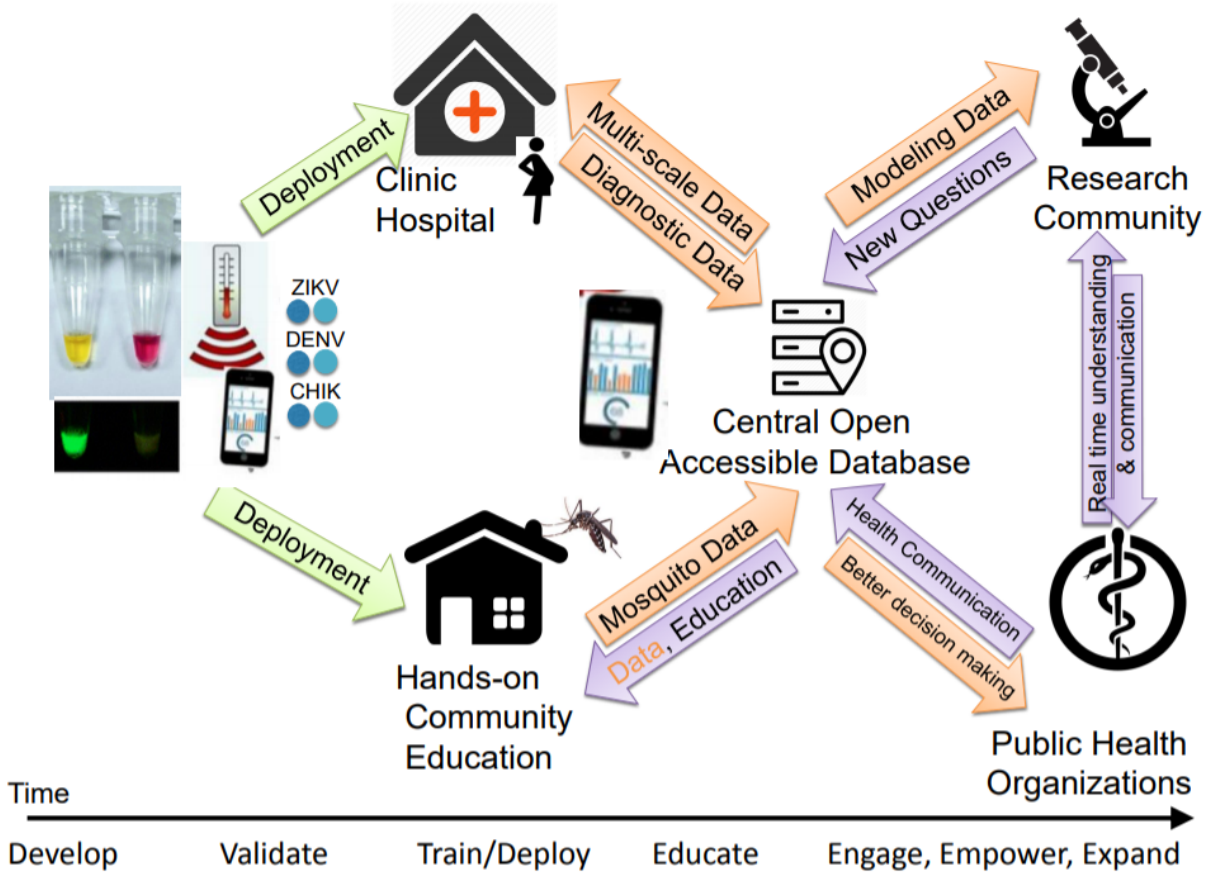


Figure 1.1.1 Original PhD proposal:Open participatory diagnostics and epidemiological surveillance of arboviruses in latin america

The initial plan was to develop open participatory diagnostics and epidemiological surveillance of arboviruses in Latin America: Zika, Dengue and Chikungunya. As a technology I focused on a promising isothermal DNA amplification technique (Loop mediated isothermal amplification (LAMP)) and was successful in adapting it for robust detection of Zika Dengue and Chikungunya viruses, judged by spiking with known quantities of viruses into plasma saliva and urine samples and compared to rt-PCR. I spent most of my 1st PhD year working towards this goal (in collaboration with Dr. Jessica Vanhomwegen, Institut Pasteur). However, concurrently a number

of similar papers were published. The difficulties associated with the high level biosecurity required to work with these viruses and the timelines and costs associated with acquiring human biofluids, infected mosquitoes and conducting international multicenter clinical trials, caused me to doubt the possibility of getting this into the hands of people during a short three year PhD.

This caused us to reassess and change directions, and as my interest was always mostly on the educational and empowering aspects this work could achieve, I decided to focus on something simpler to try and show that lay people can do genetic testing themselves. I sought something which was based on DNA not RNA, did not require biosecurity concerns, or the need for large clinical trials, but which would be interesting, engaging, fun and would allow people to engage and learn Biology in a meaningful way. This brought us to the detection of GMOs in food and feed as a first target.

1.2 Context

1.2.1 Democratization of Science

Although amateurs have been involved in the scientific endeavor even before the word scientist existed (Ross, 1962), we are in the midst of a rapid rise in so called “Citizen Science" (Brouwer et al., 2018; Cohn, 2008; Contreras-Naranjo et al., 2016; Follett & Strezov, 2015; Kullenberg & Kasperowski, 2016). Attributed particularly to the ubiquity of smartphones and the internet, coupled to growth in education (especially higher), increased leisure time, as well as the availability of cheap sensors that can collect data from the environment (Haklay, 2015). We are seeing even more people actively involved in science, by sensing, computing, analyzing, self-reporting, and/or making (Strasser et al., 2018). Important examples of these epistemic practices include; Reporting observations of hundreds of millions of birds and other wildlife on all continents (using platforms such as ebird and iNaturalist (*Cornell Lab of Ornithology—Home*, n.d.; Seltzer, 2019). Volunteering of computing resources in projects such as SETI@home and Rosetta@home helping to analyze Search for Extra Terrestrial Intelligence and protein structure data respectively.

Actively analyzing smaller pieces of large datasets such as in Galaxy Zoo and the Foldit project (shape of galaxies and 3D structure of proteins (Lintott et al., 2008; *Solve Puzzles for Science*, n.d.)). Self-reporting and sharing of medical information and experiences on platforms such as PatientsLikeMe and Open Humans (Frost & Massagli, 2008; Greshake Tzovaras et al., 2019). And finally Making (discussed below) such as in the whole so-called “do-it-yourself biology” (DIYbio) movement (*DIYbio*, n.d.) and building open source tools for environmental monitoring (Public Lab contributors, n.d.). Citizen Science is now being done in all areas of science from zoology and biology to astronomy and chemistry, which include projects related to climate change, invasive species, conservation biology, ecological restoration, water quality monitoring, population ecology and more (Bonney et al., 2016; Hulick, 2019; Silvertown, 2009).

In addition Citizen Science can also increase public engagement and scientific literacy (Land-Zandstra et al., 2016), affect changes in behavior in communities (Rhodes et al., 2010), and give people hands-on, learning by doing, face-to-face experience with science (*Citizen Cyberlab*, n.d., *SciStarter*, n.d.), in both formal and informal settings (Shah & Martinez, 2016; Zoellick et al., 2012). This gave rise to participatory or community science where people are more actively involved in all research stages, including problem/question definition (*CitizenSalmon*, n.d.; Haklay, 2013; Rhodes et al., 2010).

In recent decades we have also seen the rise of the **Maker** Movement and do-it-yourself and do-it-together (DIY & DIT) cultures (Brown & Davison, 2010; Dougherty, 2012), with thousands of FabLabs (Gershenfeld, 2005), Makerspaces and Hackerspaces (van Holm, 2014) created all over the world (fabfoundation.org). This movement is fueled by the dramatic drop in prices of digital fabrication tools such as laser cutters 3D printers and open source hardware such as microcontrollers (Blikstein & Krannich, 2013) and spurred largely by the success of Make magazine (http://makezine.com), Maker Faires (http://makerfaire.com) and an international network of FabLabs (https://www.fablabs.io/). Roughly described as community workshops where groups and individuals come together to create physical objects, these places have become powerful centers of learning and empowerment in schools (Blikstein, 2018; Eriksson et al., 2018; Peppler et al., 2016) universities,

(Weinmann, 2014; A. Wong & Partridge, 2016), libraries (Moorefield-Lang, 2015; Slatter & Howard, 2013; Willingham & de Boer, 2015) and communities (Ensign & Leupold, 2018; Taylor et al., 2016); (where) "*makers are drawn together by a shared delight in the magic of tinkering, hacking, creating, and reusing materials and technology.* (characterized by) *...deep engagement with content, experimentation, exploration, problem-solving, collaboration, and learning to learn..." (Honey & Kanter, 2013)*.

Citizen Science along with Making and **Frugal** innovation and Science (Prakash, 2018; Radjou & Prabhu, 2015; Weyrauch & Herstatt, 2016) are conjointly facilitating the democratization of science tools, making scientific equipment and experimentation cheaper, more available, more mobile, more usable (Landrain et al., 2013). Examples include microscopes; foldscopes (Cybulski et al., 2014) for less than a dollar, the 100$ FlyPi for fluorescence microscopy, optogenetics behavioral studies (Andre Maia Chagas et al., 2017) and (Nuñez et al., 2017) a multi-fluorescence imaging system for teaching and research. Open source tools for environmental monitoring of air, water and land have been collaboratively developed, shared, and sold by Public Labs (Public Lab contributors, n.d.). Making of low cost and open source neuroscience educational equipment (*Backyard Brains*, n.d.; Marzullo & Gage, 2012). Free and Open Source (scientific and medical) Hardware (FOSH) (Baden et al., 2015; André Maia Chagas, 2018; Andre Maia Chagas et al., 2020) communities, gatherings, journals and initiatives such as the Gathering for Open Science Hardware (GOSH http://openhardware.science/ and forum), PLOS Open Source Toolkit (https://channels.plos.org/open-source-toolkit), The Journal of Open Hardware (JOH), and Biomaker (*Biomaker.org*, n.d.) are being created and expanding rapidly. Similar frugal open source innovations have also been applied to molecular biology; Such as with centrifuges based on old hard disks (*Developing a Low-Cost Microcentrifuge Using E-Waste*, n.d.) or commercial drills (*DremelFuge - A One-Piece Centrifuge for Rotary Tools by Cathalgarvey*, n.d.), salad spinners to spin down 96/384 well plates (Morán & Galindo, 2011; Motohashi, 2020) , a 3D printed vortex mixer (Patel et al., 2018) and even advanced liquid handling robots (Gome et al., 2019; May, 2019; *Opentrons*, n.d.). This coupled to open protocols ((*OpenWetWare*, n.d.), protocols.io (Teytelman et al., 2016)), and even Free Genes (*Free Genes*, n.d.), Enzymes (*Open Enzyme Collection – Open Bioeconomy Lab*,

n.d.) and a legal framework for sharing materials (OpenMTA (Kahl et al., 2018)) are all coming together to help democratize molecular biology.

1.2.2 Democratization of Biology

Intertwining and building both within and upon all these recent phenomena, is the budding field of DIYbio (or 'do-it together-DITbio',Community Biology, Distributed Biotechnology) (Delfanti, 2017; Grushkin et al., 2013; Keulartz & van den Belt, 2016; Kuznetsov et al., 2015; Landrain et al., 2013). With the falling cost of equipment and the growth in knowledge of (especially molecular) biology, along with the urge for democratization of Science in general, we are witnessing this rise, with hundreds of community labs, biohackerspaces, meetups, projects and initiatives being created on all continents (*DIYbiosphere*, n.d.). Although people who engage in 'DIY/DIT bio' are heterogeneous and have various interests and motivation such as tinkering, having fun, creating or experimenting (Delfanti, 2011; Meyer, 2013), large groups have come together to generate codes of ethics (https://diybio.org/codes/) and a statement of shared purpose: (is to) "Fundamentally transform life sciences & democratize biotechnology, to inspire creativity and improve lives by organizing life science change-makers and bioenthusiasts to build an inclusive global network, cultivate an accessible commons of knowledge and resources, launch community labs and projects, and enable local educators. " (https://www.biosummit.org/). Prominent collaborative projects include making vegan cheese (https://realvegancheese.org/), teams working on new, simpler, less expensive ways to make insulin (https://openinsulin.org/), and hundreds of various art, design, technological and educational initiatives (e.g. https://www.hackteria.org/wiki/Collection_of_DIY_Biology,_Open_Source_Art_Projects).

This DIYbio movement, it has been argued, " *...could serve as a laboratory for reformulating a relationship between science and society that is less about capital accumulation and more about knowledge creation premised on participation and justice.* " (Ikemoto, 2017) and *"holds the potential to make synthetic and molecular biology more accessible and, in doing so, ... to empower citizens and to reshape the relationships between biology and society"* (Landrain et al., 2013). These

relationships interwoven with other technologies (like Artificial Intelligence) seem likely to be the central themes and issues of the 21st century (Harari, 2018).

1.2.3 Problems addressed

It is clear that the future of our children will be marked by the development of two scientific disciplines: computing and biotechnology. Regarding the first, during recent years considerable progress has been done in many countries around the globe aimed at enhancing the teaching in these subjects and, at the same time, encouraging the use of computers in classrooms. On the contrary, training in biotechnology is absent in many secondary schools around the planet. This formative deficiency generates citizens whose opinion on the marketing of biotechnological products is easily manipulated by both defenders and opposers of biotechnology. This situation is of particular relevance when the item in question is food biotechnological applications that provoke an intense social debate and more specifically the so-called genetically modified foods (GM foods). **From:** Ramón, Daniel, Alicia Diamante, and María Dolores Calvo. 2008. "**Food Biotechnology and Education**."

These questions and problems posed remain as relevant if not more so today. For the last decades understanding of biology has been growing at a rapid pace. Since the discovery of DNA, scientists have studied thousands of different genes and sequenced trillions of base pairs. We have also learned to write DNA, and can today more easily than ever make changes in genes and organisms (Synthetic Biology (Andrianantoandro et al., 2006; Benner & Sismour, 2005; Guet et al., 2002; Hutchison et al., 2016; Khalil & Collins, 2010; Richardson et al., 2017; Wurtzel et al., 2019)). But these understandings and tools are still mostly limited to academic and medical laboratories, and are relatively expensive and difficult, as has been shown by recent events and the sudden intense realization of millions of people to the existence and limitation of current common nucleic acid detection methods.

This thesis aims at asking how we can increase the availability, affordability and ease to see and detect specific sequences of nucleic acids and how we can use this as a tool to demystify, empower, educate and inspire, especially in schools, but also more generally. The choice to use GMOs was mainly because of the intense public interest for different groups worldwide, and also it is the only widely available example of new bioengineering. I believe this is an especially impactful area for

public engagement with science, both in a hands on, learning by doing and seeing way, and to promote dialogue, empowerment, and curiosity (Palmer et al., 2016; K. Todd et al., 2018).

1.2.4 GMOs and society

We decided that our first use case will be testing for GMOs in food and feed. We have seen a rapid rise in the use of GMO crops in the last 20 years, ~113-fold from 1.7 million hectares in 1996 to 191.7 million hectares in 2018 (around 15% of the world's planted cropland) , mostly in the Americas (USA Brazil and Argentina) (National Academies of Sciences, Engineering, and Medicine et al., 2017). Such that more than half of the maize, soybean cotton and canola planted worldwide are modified (Kamle et al., 2017). Most of these crops have been modified for insect resistance and/or herbicide tolerance, but more recent authorized strains include modifications for disease resistance, anti-allergy, delayed fruit softening/darkening, modified oil/fatty acid content, and drought resistance (James, 2017) Future crops are being engineered for improved photosynthesis (Simkin et al., 2019; Weber & Bar-Even, 2019), improved carbon dioxide (CO2) capture and storage (*Harnessing Plants Initiative*, n.d.) detoxification of cotton seeds (Rathore et al., 2012), improved yields and grain size (X. Li et al., 2020) as well as improved resistance to salinity, nutrient content (biofortification) and tolerance to biotic/abiotic stresses (*GMO Crops Have Been Increasing Yield for 20 Years, with More Progress Ahead*, n.d.). Although, since their inception there has been an ongoing debate (Gerasimova, 2016) on their use and adoption around the world and in different countries, with only 26 countries allowing planting and another 44 allowing import for food, feed, and processing in 2018 (James, 2017). There are also differences between countries in the information available to consumers, with only 65 countries requiring labeling of some sort (*How Are GMOs Labeled around the World? #GMOFAQ*, 2016).

More than 10 years before the first plant was genetically modified (Bevan et al., 1983), just as scientists were first learning how to "cut and paste" DNA from different organisms, controversy had already started (see: (Berg & Singer, 1995). A moratorium on certain types of experiments was put in place, which received considerable media coverage and prompted the National Institutes of Health (NIH) to

establish the first Recombinant DNA Advisory Committee in October 1974 (Lenzi et al., 2014). A related conference the next year was mostly concerned with safety issues, which concluded that appropriate safeguards and containment of the newly created organisms should be implemented and involved not only scientists but also lawyers, members of the press and government officials (Barinaga, 2000). From 1976 onwards, DNA manipulation changed from an only academic and fundamental research discipline, to a also being commercial, altering attitudes and research emphases; from only scientific values, towards also including economic ones (Devos et al., 2008; Welsh & Glenna, 2006). Commercialization inspired societal concerns regarding not only safety in labs but also environmental aspects, consumption, and employment, which led to worldwide adoption of Technology Assessment (TA) initiatives, responsible for advising policy makers and the public on the potential impacts of new technologies and their societal concerns (Genus & Coles, 2005). Recombinant DNA Safety Considerations: Safety Considerations for Industrial, Agricultural and Environmental Applications of Organisms Derived by Recombinant DNA Techniques. (Oecd, 1986) (commonly referred to as the "Blue book") was the first intergovernmental document addressing these issues, and contributed to the achievement of international consensus on using case-by-case and step-by-step procedure in environmental risk assessment (Schiemann, 2006).

The first human gene therapy experiments failed and were done in opposition to NIH guidelines, and without the approval of the Institutional Review Board (e.g. the Cline affair see: (Beutler, 2001; Dickson, 1981)[1]. Raising outcries and serious ethical concerns yet again, along with questions about premature experiments, irreversible manipulations of human genes, patient rights, and the responsibilities of researchers. The first transgenic and cloned large mammals (e.g., Herman the bull, Tracy and Dolly the sheep) also caused significant ethical controversies, mainly due to animal health and welfare concerns, but also in respect to the intrinsic value of animals, or to animal "integrity" or "dignity" (Blackford, 2016; Ormandy et al., 2011). These debates were explicitly labeled "Bioethical" and spurred the "institutionalization of

[1] Not at all unlike the recent unapproved experiments using CRISPR for human embryo germline editing in China (e.g. the He Jiankui affair see: (Kirksey, 2020; Krimsky, 2019) which have lead to prison sentences (Cyranoski, 2020) and a new call for a moratorium on heritable human genome editing (Lander et al., 2019).

ethics", establishing ethics committees at the hospital, academic, local and national levels, with regulation encompassing moral and ethical principles (Kamara et al., 2001; Vàzquez-Salat et al., 2012).

The introduction of genetically engineered agro-food products into the public sphere began in the mid nineties, which caused little public concern in the USA, whereas the opposite was true in Europe. This difference has been attributed to numerous reasons; such as different attitudes toward food and eating, technological mastery of land, reliability of scientific experts, or the value of local traditions and agriculture (Ujj, 2016). In addition to previous legitimate safety related scares, such as bovine spongiform encephalopathy (BSE or mad cow disease), HIV-contaminated blood, asbestos, and other perceived regulatory failures which had eroded European's trust in industry and government. This together with widely publicized, controversial scientific announcements (Butler, 2012) and coordinated anti-GMO campaigns (Bauer, 2018; Devos et al., 2008). In France, various tactics have been used to contest GMOs: trashing GM crops in fields (as well as grain in silos), organizing hunger strikes, targeting food retailers and blocking ports. Locally, some leaders reframed the debate as an issue on food quality, where topics such as paysan expertise, cultural homogenization, and globalization were emphasized (Meyer, 2017). Changing a framing related to risks and scientific concerns (health problems such as allergies and cancer, overuse of pesticides and herbicides, superweeds and super insects (Daubenmire, 2019) into a "complex of concerns.. (where) any distinctions between environmental, agricultural, and socio-economic issues proved to be blurred, fueling the confusion about the wider debate about genetic modification and the risk assessment of GM crops" (Devos et al., 2008). Leading to very different public perceptions and regulatory frameworks; Policies in the US, based on the substantial equivalence principle, stand in contrast to the EU's regulations, which contain precautionary clauses on traceability, mandatory labeling and thresholds (Stephan, 2015). Across the period 1996-2010, a downward trend in the percentage of supporters of GMO foods was observed, with only around 25% support across Europe (Gaskell et al., 2010). These trends seem to have reversed with a recent poll finding that the level of reported concern has decreased

dramatically from 66% in 2010 to 27% in 2019 (*Food Safety in the EU*, 2019). In the mid-to-late 90s, in US studies, between two-thirds and three-quarters of US consumers supported biotechnology and were willing to accept GM-enhanced food (O'Connor et al., 2004), while more recent polls 39% said GM foods are worse for one's health, rising to 51% in 2019, with younger respondents being more concerned than older ones (Funk, 2020; Funk & Kennedy, 2016).

(Gerasimova, 2016) argues that the main points of contention include the effects of GM crops on the environment and human health, economics, and social development, and that both side use the same framework of sustainable development, with both sides claiming to support biodiversity, food security and poverty alleviation.The choice to use GMOs was mainly because of the intense public interest for different groups worldwide, and also it is the only widely available example of new bioengineering. I believe this is an especially impactful area for public engagement with science, both in a hands on, learning by doing and seeing way, and to promote dialogue, empowerment, and curiosity (Palmer et al., 2016; K. Todd et al., 2018).

1.3 Thesis overview

Here, I present the use and development of isothermal amplification techniques to engage students and the public with biotechnology and some of its central issues. Resulting in a shelf-stable, easy to use, affordable and effective educational kit/workshop for detecting GMOs in food or feed. After a short history of DNA, I will cover isothermal amplification techniques as an alternative to PCR, especially LAMP (Loop mediated isothermal amplification) and how I improved, optimized and adapted existing LAMP methods for GMO detection, for use by laypeople (Chapter 2). Then, I will discuss some of the technical hurdles in taking these reactions outside of a lab setting and into the "wild". These include nucleic acid extraction, access to reagents (wetware) and tools, stable isothermal heating, as well as amplification detection. I show how we overcame all these barriers, while lowering the price of each step by at least an order of magnitude. This was all packaged into a modular workshop for laypeople and students, which was iterated upon and improved (Chapter 3). Next I discuss hands-on learning activities in general, and in

molecular biology in particular in both formal (school) and informal settings. I show how we implemented the developed workshop and methodology in a variety of contexts and its effectiveness in improving understanding, interest, skill level and empowerment (Chapter 4). Finally, I conclude by discussing this work in a wider frame and give prospective avenues for further work and research (Chapter 5).

Chapter 2:

Developing a robust assay to detect common transgenic elements

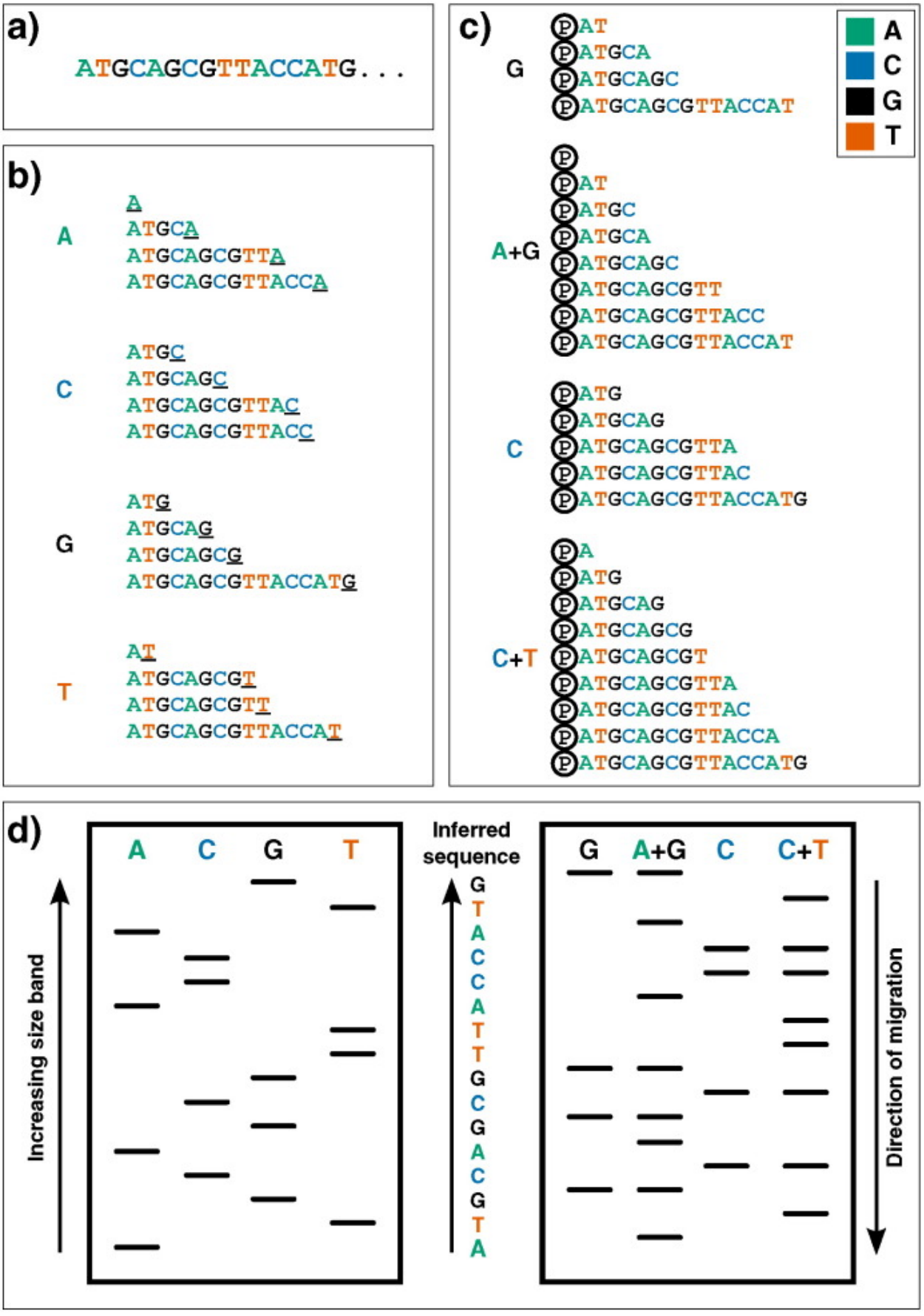
2.1 Introduction

2.1.1 A short history of DNA:

Modern understanding of genes and DNA has been growing since the first discovery of nucleic acid a century and a half ago. Just 3 years after Augustinian monk Gregor Mendel published his work on the patterns of inheritance in pea plants, in the late 1860s, a young swiss doctor named Friedrich Miescher, was the first person to isolate phosphate-rich chemicals from white blood cells. He named these chemicals (which would eventually be known as RNA and DNA) nuclein because they were isolated from the nuclei of the cells (Dahm, 2008; Dahm & Banerjee, 2019; Miescher, 1897). It would take another 75 years until Oswald Avery and colleagues identified DNA as the 'transforming principle'. Avery worked with the bacterium responsible for pneumonia, pneumococcus, and had discovered that if a live, but harmless form of pneumococcus was mixed with an inert but lethal form, the harmless bacteria would soon become deadly. He isolated proteins, carbohydrates, nucleic acids and applied them to non-virulent bacteria. Only nucleic acids (DNA) caused the change (Avery et al., 1944). In 1950, Erwin Chargaff published his two major findings: first, that in any double-stranded DNA, the concentrations of thymine and adenine, and cytosine and guanine, are always found in equal amounts, and that nucleotide composition of DNA varies among species (Chargaff, 1950). This lead to the well-known discovery by James Watson and Francis Crick, with contributions from Rosalind Franklin and Maurice Wilkins, of the double helix structure of DNA (Watson & Crick, 1953). Their

model of the structure of DNA fulfilled the characteristics necessary for the genetic material: auto-replication, specificity, information content and the ability of the genes to mutate (Portin, 2014).

Subsequent studies suggested the central dogma of biology where DNA gets transcribed in mRNA which in turn is translated into protein (Crick, 1958; Jacob & Monod, 1961). This led Marshall Nirenberg, Har Gobind Khorana, and colleagues, to crack the ‘code for life’. They identified how the letters in DNA are read in blocks of three called a codon. Each codon specifies an amino acid that is added to the protein during synthesis(Nirenberg et al., 1965). The genetic code was shown to be practically universal, shared by all organisms on earth, be they a virus, bacterium, archaea, fungus, plant or animal (Portin, 2014). These developments generated a thirst for reading the nucleotides in DNA molecules, what we now call sequencing. The first methods were developed in the 70s, improved versions of which were published together in 1977 (Maxam & Gilbert, 1977; Sanger et al., 1977). These methods were based on running radioactively labeled DNA molecules on a high-resolution polyacrylamide gel and then reading “up” the gel. In the sanger method, the different fragments are created by adding low amounts of a specific nucleotide which ends a synthesis reaction, whereas in the Maxam and Gilbert method the DNA molecules are cleaved at specific bases depending on the chemicals used. (See **Fig 2.1.1**)


a)
ATGCAGCGTTACCATG...
b)
A
ATGCA
ATGCAGCGTTA
ATGCAGCGTTACCA
C
ATGC
ATGCAGC
ATGCAGCGTTAC
ATGCAGCGTTACC
G
ATG
ATGCAG
ATGCAGCG
ATGCAGCGTTACCATG
T
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ATGCAGCGT
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ATGCAGCGTTACCAT
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ATGCAG
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ATGCAGCGTTAC
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ATGCAGCGTTAC
ATGCAGCGTTACCA
ATGCAGCGTTACCATG
d)
A
C
G
T
Increasing size band
Inferred sequence
G
T
A
C
C
A
T
T
G
C
G
A
C
G
T
A
G
A+G
C
C+T
Direction of migration

Figure 2.1.1 First-generation DNA sequencing technologies. Example DNA to be sequenced (a) is illustrated undergoing either Sanger (b) or Maxam–Gilbert (c) sequencing. (b): Sanger's 'chain-termination' sequencing. Radio- or fluorescently-labeled nucleotides of a given type - which once incorporated, prevent further extension - are included in DNA polymerization reactions (c): Maxam and Gilbert's 'chemical sequencing' method. DNA must first be labeled, typically by inclusion of radioactive P 32 in its 5′ phosphate moiety (shown here by Ⓟ). Different chemical treatments are then used to selectively remove the base from a small proportion of DNA sites. (d): Fragments generated from either methodology can then be visualized via electrophoresis on a high-resolution polyacrylamide gel: sequences are then inferred by reading 'up' the gel, as the shorter DNA fragments migrate fastest. In Sanger sequencing (left) the sequence is inferred by finding the lane in which the band is present for a given site, as the 3′ terminating labeled nucleotide corresponds to the base at that position. Maxam–Gilbert sequencing requires a small additional logical step: Ts and As can be directly inferred from a band in the pyrimidine or purine lanes respectively, while G and C are indicated by the presence of dual bands in the G and A + G lanes, or C and C + T lanes respectively. (adapted from (Heather & Chain, 2016) with permission)

Even before this work, the first DNA splicing was conducted. Using newly discovered enzymes which could "cut and paste" DNA (restriction enzymes and ligases (Arber & Linn, 1969; Heinrichs, 2007; Lehman, 1974; Meselson & Yuan, 1968; Sadowski et al., 1968; Zimmerman et al., 1967), Berg and colleagues combined genes from a virus and a phage into one molecule (Jackson et al., 1972). Boyer and Cohen took this one step further and took genes from other bacteria and even a frog gene, and spliced them into bacterial plasmids, which then replicated and were functional in *E.coli (Cohen et al., 1973; Morrow et al., 1974)*. A year after that the first genetically modified mouse was reported (Jaenisch & Mintz, 1974) followed by the Asilomar moratorium on genetic engineering research ("Potential Biohazards of Recombinant DNA Molecules," 1974) until the implications of this line of research could be more thoroughly discussed (Berg et al., 1975).

Another monumental change in molecular biology and the study of DNA came with the invention of the Polymerase Chain Reaction (PCR) in the mid-1980s (Bartlett & Stirling, 2003). Prior to this, in order to create large amounts of a specific DNA sequence, one had to clone it into a plasmid and transform it into a bacteria which would then create more copies of the plasmid as the bacteria doubled. PCR allows

specific amplification of a target sequence rapidly and easily (Mullis et al., 1986; Saiki et al., 1985). Briefly, two short oligonucleotide "primers" are designed to be complementary to the edges of the region which we want to amplify from a DNA target. These are mixed along with a thermally stable DNA polymerase (Chien et al., 1976; Saiki et al., 1988), dNTPs (deoxynucleotide triphosphates, nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand; a buffer solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase; bivalent cations, typically magnesium (Mg) and monovalent cations, typically potassium (K) ions. The reaction is then typically cycled through three different temperatures; first, it is heated to ~95 °C to denature the double-stranded DNA (**Fig 2.1.2 ❶**). The second step involves cooling the reaction to ~60 °C in order for the primers to anneal to their complementary strand through base-pairing interactions (**Fig 2.1.2 ❷**). This is followed by a third step at ~72 °C at which the polymerase elongates the primers generating a new copy of double-stranded DNA (**Fig 2.1.2 ❸**). These cycles are repeated ~30-40 times, each cycle, in theory, doubles the amount of DNA thus 30 cycles create 2^{30} (~1 billion) copies of the target stretch of DNA (**Fig 2.1.2 ❶❷❸**).

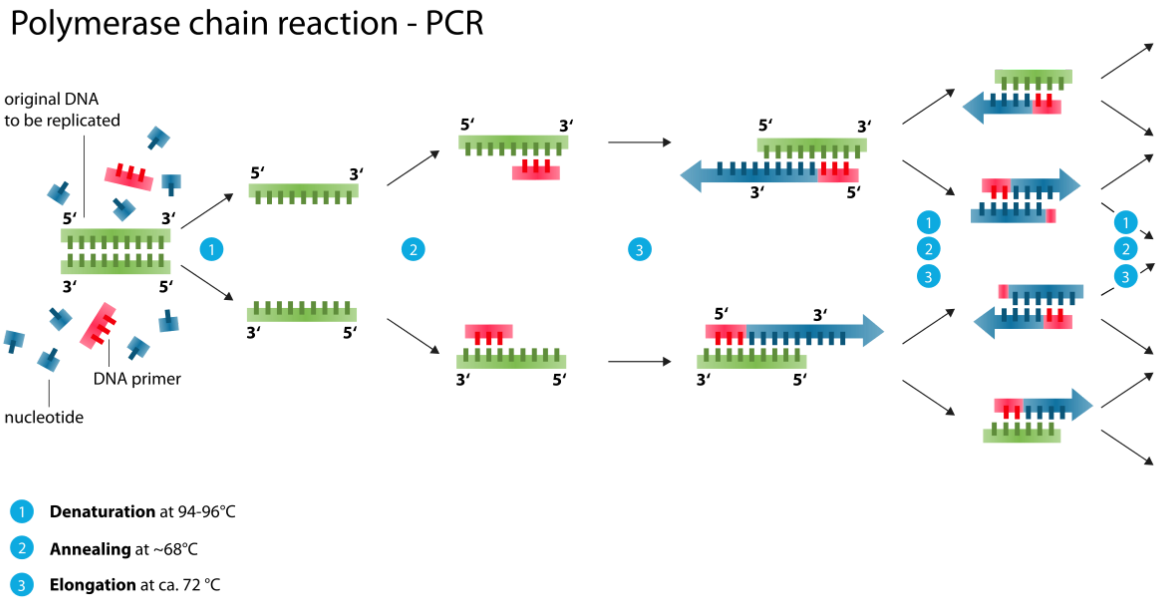

Figure 2.1.2 Polymerase Chain Reaction (PCR) This process includes 30–40 cycles the following steps: ❶) Denaturation of DNA template by heat; ❷) Primer annealing to denatured targeted DNA at a specific temperature; ❸) DNA synthesis by a thermostable DNA polymerase (© User:Enzoklop / Wikimedia Commons / CC-BY-SA-3.0)

This simple and rapid method was quickly adopted by scientists and became ubiquitous in all molecular biology labs around the world (Bartlett & Stirling, 2003; Dove, 2018) (**Fig 2.1.3**). Contributing to this rapid rise were amongst others; The invention and commercialization of thermocyclers which can heat and cool sample tubes rapidly, at precise temperatures for specific periods of time (Dove, 2018; Weier & Gray, 1988). The discovery and improvement of a variety of thermostable DNA polymerases improving aspects of the reaction such as accuracy, speed, and length of the amplicons (Ishino & Ishino, 2014). And the development of real-time or quantitative PCR, which allows precise quantification of the amount of DNA or RNA in a sample, by monitoring the reaction while it is happening and comparing this to similar reactions with known amounts of template (Bustin et al., 2009; Higuchi et al., 1993).

Such that today it is the most widely used method of DNA amplification for detection and identification of infectious diseases, genetic disorders and other research purposes(Gill & Ghaemi, 2008). But this method also has some drawbacks, especially for use by laypeople and outside of a laboratory setting as will be discussed in section following sections and chapters in detail.

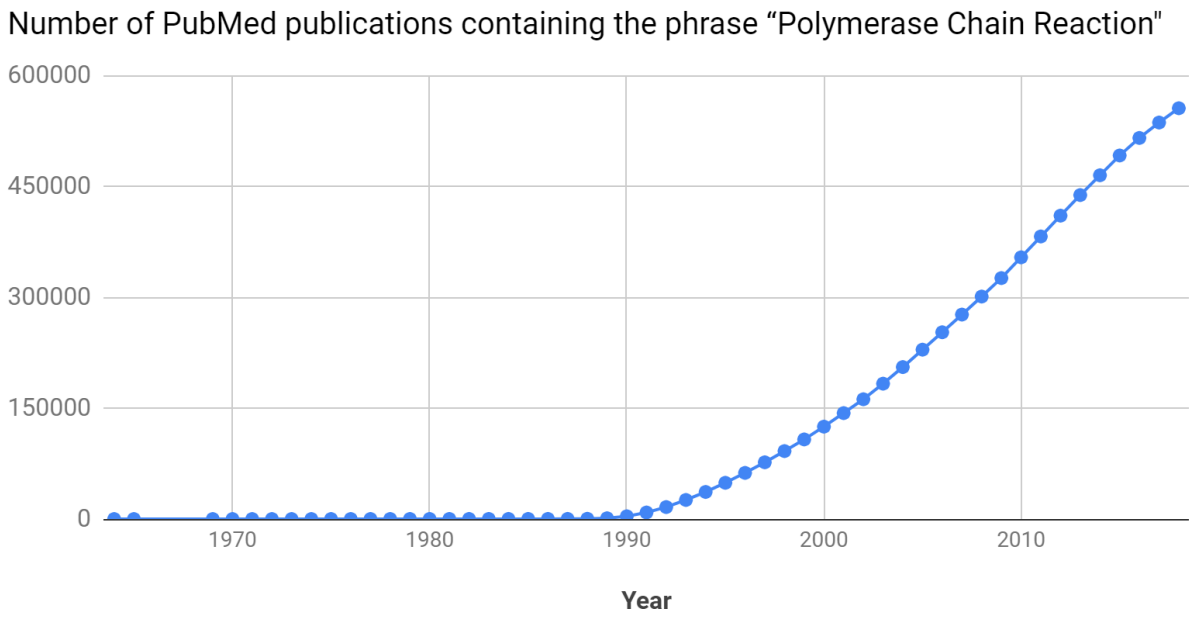

Figure 2.1.3 Cumulative number of PubMed articles containing the phrase “Polymerase Chain reaction”. (PubMed search, Results by year).

These improvements of PCR allowed the rise of so-called next generation sequencing (NGS) or second generation sequencing (McGinn & Gut, 2013; Metzker, 2010). Rather than creating many copies of the DNA to be sequenced using bacterial cloning, in vitro amplification generates multiple copies of each template to be sequenced, in a massively parallel fashion on a two dimensional surface all in a single chamber (**Fig 2.1.4.** amplification). Also instead of measuring fragment lengths, NGS comprises cycles of biochemistry and measurement, also known as ‘sequencing-by-synthesis’ (SBS). The detection can be done either by chemiluminescence, with pH changes or the most common being fluorescence on the Illumina platform (McGinn & Gut, 2013; Shendure et al., 2017).

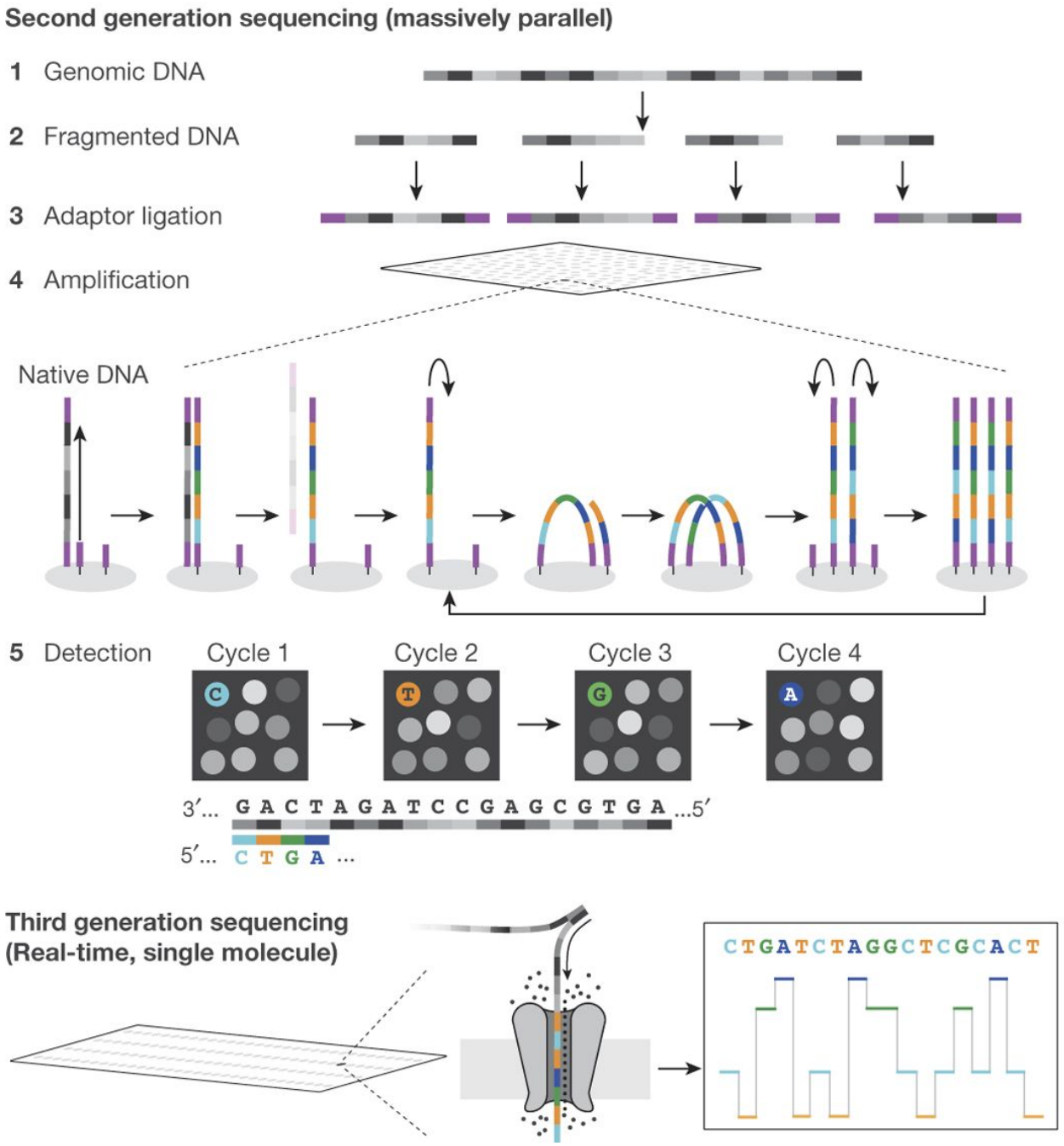

Figure 2.1.4 Evolution of DNA sequencing methods: Second generation **above)** DNA is broken into fragments which are attached to adapters that connect them to a flow cell. These are then copied locally through 'bridge amplification'. Sequencing itself is achieved in a SBS manner using fluorescent 'reversible-terminator' dNTPs, which cannot immediately bind further nucleotides until they are cleaved either enzymatically or with light. These modified dNTPs are washed over the primed, single-stranded flow-cell bound clusters in cycles. At each cycle, the identity of the incorporated nucleotide can be monitored. Third generation **below)** DNA is placed in a flow cell above a membrane arrayed with nanopores. The DNA then passes through a biological nanopore embedded in a membrane, across which a voltage is applied. As the ssDNA passes through the nanopore the different bases prevent ionic flow in a distinctive manner, allowing the sequence of the molecule to be inferred by monitoring the current at each channel. Adapted with permission from (Heather & Chain, 2016; Shendure et al., 2017)

We are currently seeing the beginning of a rise in third generation sequencing which allows real-time reading of long single molecules without the need for prior amplification (McGinn & Gut, 2013). The most common of these is nanopore sequencing in which the identity of nucleic acids is inferred from changes in the ionic current across a membrane as a single DNA molecule passes through a protein nanopore such as in the MinION from Oxford Nanopore (Jain et al., 2016) (**Fig 2.1.4**). A significant advantage of these methods is that they do not require optical systems, thus allowing them to be much smaller lighter and mobile; eg the MinION sequencing system is smaller than a phone connects straight to a USB hub and weighs only 90 grams which is useful for field applications (Kono & Arakawa, 2019). This also allows real-time reading of the sequencing as it is happening without the need to wait for a whole run to finish as in older technologies, which could take days. Lastly, these systems are also relatively affordable with the hardware starting at 1000$ and consumables (a flow cell that can be reused a few times costing ~500$ and library preparation kits costing ~100$) (*Product Comparison*, n.d.). Unfortunately, this technology is still too immature, complex and expensive for use by laypeople.

2.1.2 Isothermal amplification techniques as an alternative to PCR

Although PCR is widely adopted, developed and used it has a variety of shortcomings, especially for our purposes. PCR requires expensive and precise equipment (thermocycler), which even an open-source DIY version costs 500$ and has a very low throughput (8 reactions at a time) (*OpenPCR - the $499 Open Source PCR Machine / Thermal Cycler*, n.d.). Similar commercial versions aimed at the educational market start at 1000$ (miniPCR(miniPCR Team, 2018)), while versions able to process more samples cost thousands. A standard PCR is slow (at least 3 hours per experiment) additionally, PCR is sensitive to inhibitors so usually requires costly, complicated and time-consuming DNA extraction (and clean up) (Fakruddin et al., 2013). Finally to get results from a PCR experiment usually requires running the PCR product in an agarose gel, which is not trivial to interpret, and is also lengthy, not very simple to do, and requires more equipment, temperatures, high voltages and DNA binding dyes (**Fig 2.1.5)**. Here I chose to focus on a newer **Isothermal**

DNA amplification technology that can overcome some of these drawbacks (Gill & Ghaemi, 2008; Zhao et al., 2015).

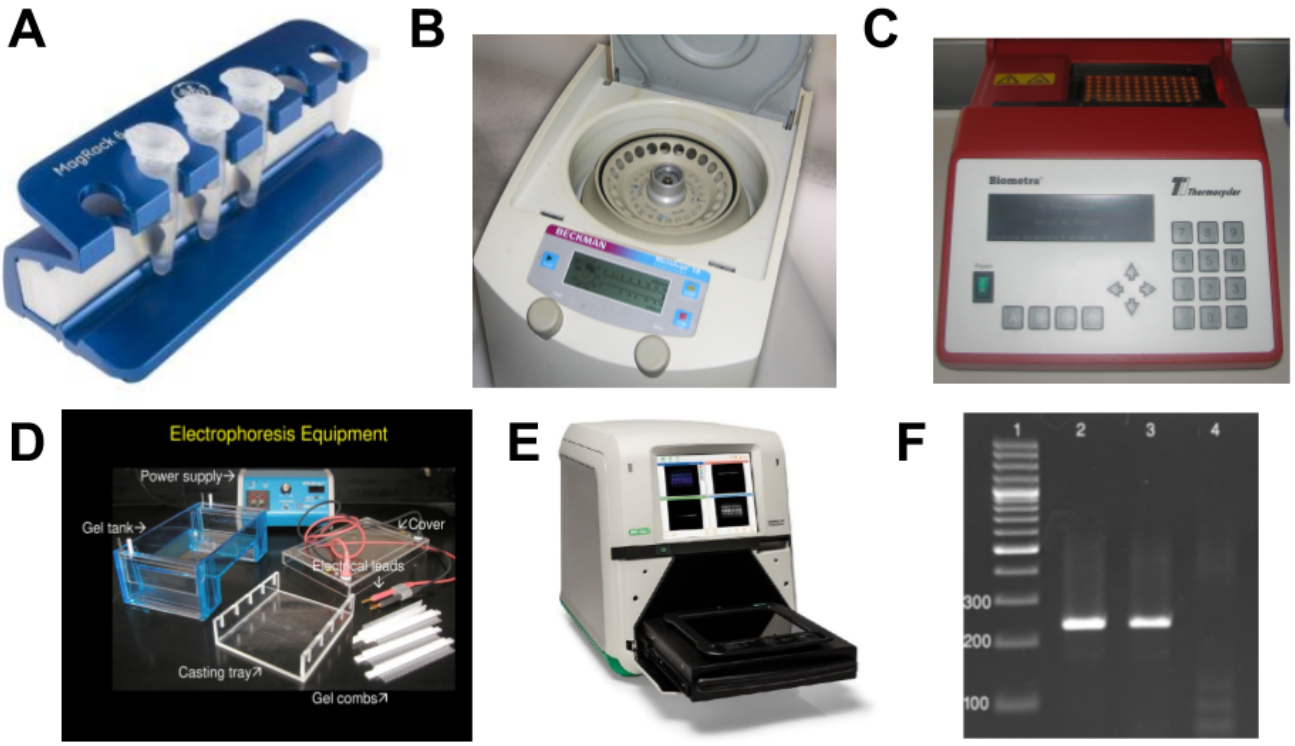

Figure 2.1.5 PCR and related equipment A) Magnetic rack and B) Microtube centrifuge, which are required to remove PCR inhibitors and extract nucleic acids. C) Thermocycler. D) Electrophoresis equipment including: High voltage power supply, cables, Gel tank and cover, Casting tray and gel combs. E) Gel imager. F) PCR Gel results.

These shortcomings created the need for simpler nucleic acid amplification and detection techniques, which do not require thermal cycling, hence isothermic. Since the early 1990s, dozens of isothermal nucleic acid amplification techniques employing various mechanisms have been developed. Most of these methods possess impressive sensitivity for the detection of nucleic acids. These methods can be broadly categorized into four different groups based on: RNA transcription, DNA replication with enzymatic duplex melting/primer annealing, strand-displacing polymerization from inherently circular targets, or strand displacement using polymerases from a linear target. These methods have promise mainly for use in low resource/field settings and for point of care diagnostics.

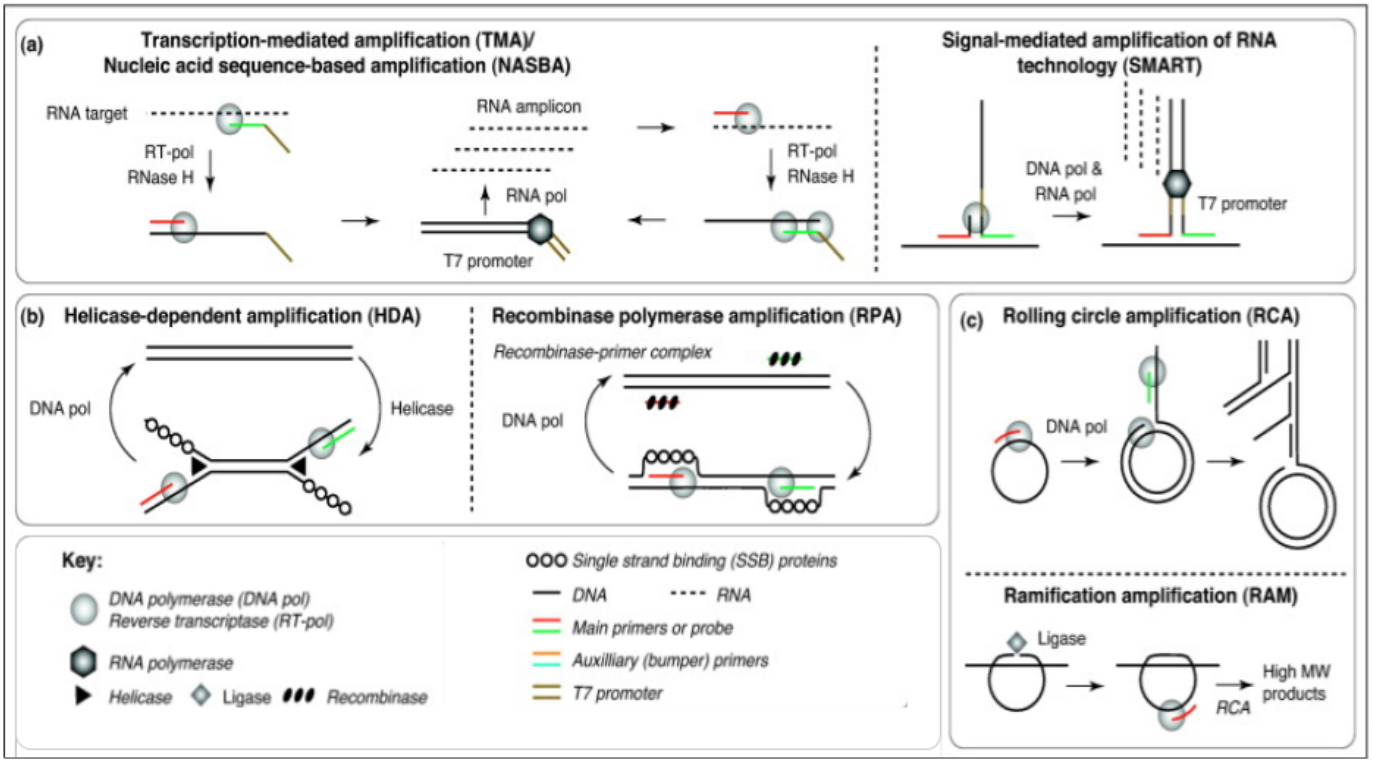

Figure 2.1.6 Overview of isothermal nucleic acid amplification reactions. a) Methods based on RNA transcription. In TMA/NASBA, an RNA target is converted to cDNA with a T7 promoter region through reverse transcription, followed by RNase H degradation of the original strand and DNA polymerization initiated by a second primer. RNA polymerase amplification creates products that feed back into the original reaction. SMART utilizes a three-way junction with target and extension probes to initiate linear RNA polymerization based amplification (no exponential feedback). b) Methods based on DNA replication with enzymatic duplex melting/primer annealing. In HDA, a helicase enzymatically ''melts'' dsDNA. In RPA, a recombinase–primer complex scans dsDNA for the target site and facilitates primer binding. In both HDA and RPA, single-strand binding proteins stabilize the separated strands; the rest of the reaction sequence is analogous to PCR. c) Methods based on strand-displacing polymerization from inherently circular targets in RCA. or from padlock probes that are circularized through the action of a ligase in RAM. In both cases, branched amplification can be initiated through a second primer. Adapted with permission from:(Niemz et al., 2011)

Nucleic Acid Sequence Based Amplification (NASBA) (**Fig 2.1.6 (a)**) is the oldest isothermal amplification method, and was specifically developed for detection of RNA (Kievits *et al*. 1991). NASBA mimics *in vivo* retroviral replication mechanisms to produce RNA amplicons from an RNA template through a cDNA intermediate. Three enzymes are involved: a reverse transcriptase (RT), RNase H and T7 DNA dependent RNA polymerase, and two primers, one of which has a T7 promoter attached, and is performed at 41 Degrees (Deiman et al., 2002). It also requires an incubation step at 65 °C to denature the target prior to the addition of enzymes.

NASBA achieves 10 million fold amplification in 1–2 h, and has been used to detect many RNA viruses such as HIV-1,' hepatitis C, enterovirus. Detection can be done using gel electrophoresis, real-time fluorescence or colorimetric assays (Yan et al., 2014). Transcription-mediated amplification (TMA) is the same reaction but requires only two enzymes, as it uses a RT-DNA polymerase that has intrinsic RNase H activity (Brentano & Mcdonough, 2000).

Signal-mediated amplification of RNA technology (SMART), also works by using a T7 promoter to transcribe an RNA signal, which only becomes active if a DNA or RNA target is present. The system utilizes two single-stranded oligonucleotide probes; an extension probe and a template probe. The extension probe is shorter in length than the template probe, the template probe consists of a single-stranded T7 RNA polymerase promoter sequence. Both probes contain one region that can hybridize to the target sequence and a second much shorter region (6–8 nucleotides) that hybridizes to create the structure of a three-way junction (3WJ) (**Fig 2.1.6 (a)**). A denaturation step (at 95 °C) is performed at the beginning of the reaction, followed by the addition of the two enzymes and isothermal amplification at 41 °C. Formation of the 3WJ allows the Bst DNA polymerase to extend the template from the end of the extension probe, which in turn generates a double-stranded T7 RNA polymerase promoter from which transcription of multiple copies of an RNA signal occurs. This method has not been used extensively as it takes 2-3 hours and generates a non-specific signal (W. Xu, 2016). These methods are inappropriate for our goals mostly because they require more than one temperature, more than one enzyme and both dNTPs and rNTPs (Yan et al., 2014).

Helicase-dependent amplification (HDA) mimics natural DNA replication and relies on using a helicase enzyme to open the double-stranded DNA which is then coated by single-stranded DNA-binding proteins. Then primers bind/hybridize and are extended allowing a polymerase chain reaction to happen at isothermal conditions (Vincent et al., 2004) (**Fig 2.1.6 (b)**). In the first version of HDA, the reaction was done at 37 °C due to the thermal instability of E. coli helicase. The use of thermostable polymerases and helicases has enabled faster amplification at higher temperatures (60−65 °C) and with improved sensitivity and specificity (An et al.,

2005). A variety of pathogens have been detected using HDA with a limit of detection as low as 10 copies, with reactions taking between 30-120 minutes.

Recombinase Polymerase Amplification (RPA) employs an enzyme (recombinase) which when combined with a primer can help to find and then bind and open the complementary double-stranded DNA. This process is aided by ssDNA binding proteins, which bind to the “melted” DNA to allow primer extension (Piepenburg et al., 2006) (**Fig 2.1.6(b)**). RPA can create billions of DNA copies within 40–60 min at incubation temperatures between 37 °C and 42 °C (J. Li & Macdonald, 2015). RPA has been growing in use in recent years to detect a plethora of pathogens down to one cell or colony-forming unit (CFU). Detection methods for both HDA and RPA include gel-electrophoresis, real-time fluorescence and lateral flow devices amongst others. (Barreda-García et al., 2018; J. Li et al., 2018). Unfortunately, these methods are proprietary and because they require 3-5 different recombinant proteins are relatively expensive. Commercial versions cost at least 4$ per reaction (NEB, TwistDX websites respectively).

Rolling-circle amplification (RCA) (Fire & Xu, 1995; Lizardi et al., 1998) **and Ramification amplification** (RAM) (D. Y. Zhang et al., 1998) are based on DNA replication of viruses with circular genomes. They require circular ssDNA in order to amplify their target and thus are useful for the detection of DNA molecules which exist in vivo as such and include plasmids and certain phage, virus, and viroid genomes(Craw & Balachandran, 2012) (**Fig 2.1.6 (c)**). In order to use this method on non-circular DNA some kind of pretreatment is needed. These usually come in the form of using a probe (padlock) which if it is complementary to both edges of a single-stranded target then recruits a ligase enzyme which then circularizes the DNA and then allows amplification (Banér et al., 1998; Mohsen & Kool, 2016). However this circularization process demands additional enzymes, procedural complexity and cost (Craw & Balachandran, 2012) and is not ideal for our intended purpose.

2.1.3 LAMP

2.1.3.1 Introduction

We chose to use Loop mediated isothermal AMPlification (LAMP) which is the most well established and developed isothermal amplification technology (W. Xu, 2016) offering rapid, accurate, single tube and cost-effective target DNA amplification (Yasuyoshi Mori & Notomi, 2009). The LAMP method was first published in 2000 (Notomi et al., 2000), in the original formulation it employs the large fragment of a thermostable DNA polymerase from *Bacillus stearothermophilus* (Bst) (Aliotta et al., 1996) with strong strand displacing activity, and 4 primers, which recognize 6 specific regions and facilitate the formation of “loop” structures, which allow the polymerase to initiate synthesis at multiple locations, at around 65℃ (Craw & Balachandran, 2012) (See **Fig 2.1.7 and 2.1.8**). LAMP can then amplify single copies of the target DNA or RNA to 10^9 in less than one hour (amplification is factorial as opposed to exponential in PCR), even when large amounts of non-target DNA are present and even with “dirty” samples (Kaneko et al., 2007). LAMP was found to be up 100 fold more sensitive than traditional PCR (Thai et al., 2004), other advantages include no need for equipment except a water bath/heat block and pipettes, and easy visible detection using turbidity, colorimetric or fluorescent assays (Mohammad Amin Almasi et al., 2013; Tanner et al., 2015; Tomita et al., 2008) (discussed in detail in the following section), making it more fitting for use by lay people than PCR. Disadvantages of LAMP compared to PCR and some other isothermal methods include; More difficult/complex primer design, amplified region must be between ~160 to ~280 base pairs long, and attention needs to be given to the melting temperature (Tm) as well as length and distance of the different primers (though software exists to facilitate this (Eiken Chemical Co. , Ltd., n.d.; Jia et al., 2019; Torres et al., 2011)). Additionally LAMP reactions are more difficult to multiplex, and LAMP reaction products are less suitable for downstream applications (i.e. cloning, sequencing) as they are long cauliflower-like concatemers of varying length and not single copies (Eiken Chemical Co. , Ltd., n.d.; *LAMP Loop-Mediated Isothermal Amplification Webinar Lucigen*, n.d.) and **Fig 2.1.8**.

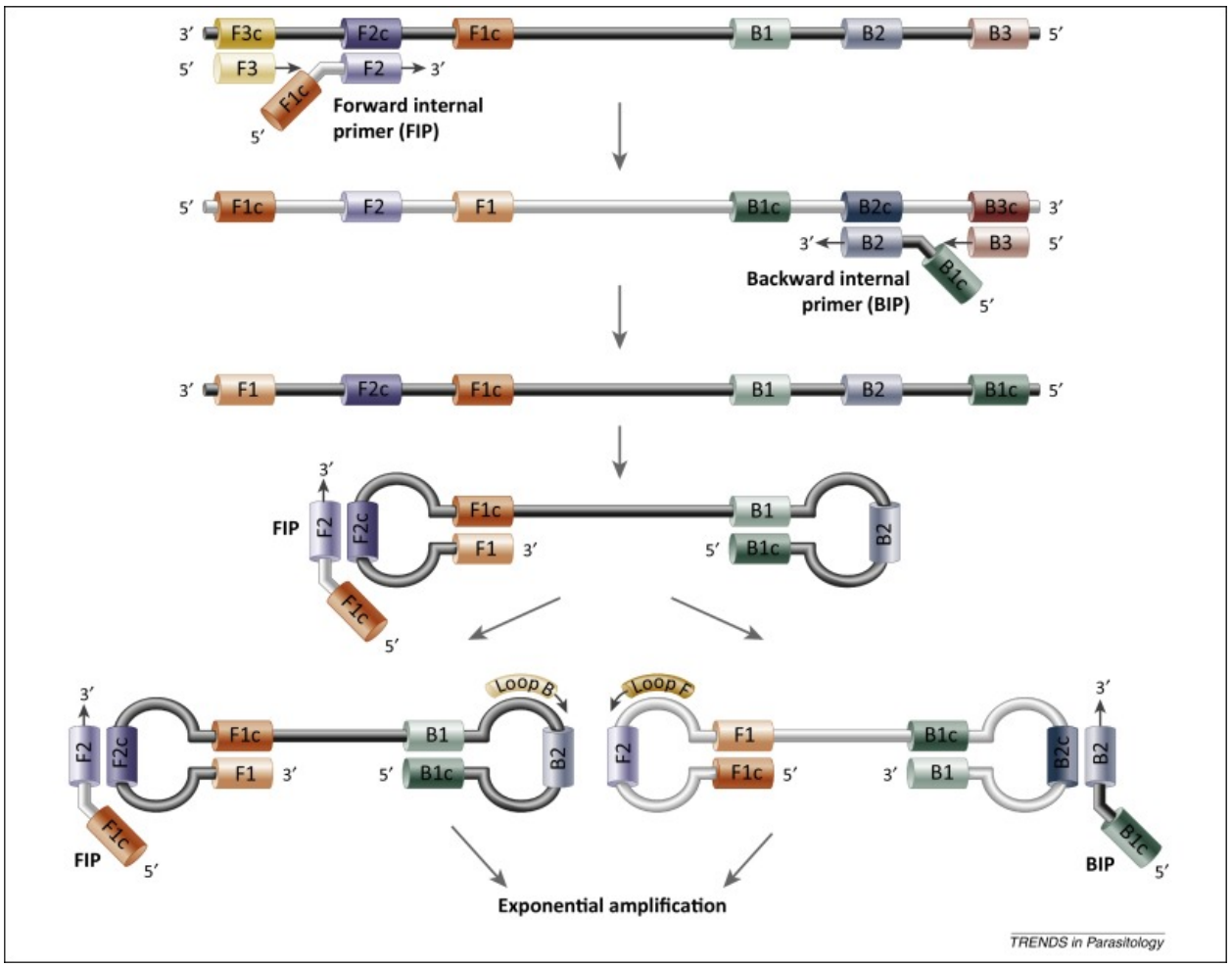

Figure 2.1.7 LAMP initiation: LAMP employs two sets of primers, forward/backward internal primers: FIP and BIP, and outer primers F3 and B3, to target six distinct regions (F1c, F2c, F3c sites on one end and B1, B2, B3 sites on the other). The reaction is initiated by the binding of FIP to the F2c region on the double-stranded DNA. As the polymerase elongates the DNA from the FIP, the outer primer F3 which is shorter in length and lower in concentration than the FIP then binds onto its complementary region on the DNA and starts to displace the newly synthesized DNA. The replaced strand then forms a loop structure at one end due to the **complementarity of F1 and F1c**. With similar performance of BIP and B3, this results in a single-stranded double stem-loop DNA structure (so-called "dumb-bell" structure). This dumbbell structured DNA enters the amplification cycle as it is already self-primed and elongation by the polymerase can happen from the free 3' end of the ssDNA and from binding of the FIP/BIP primers to the single stranded loop or from the optional accelerating loop primers (See next figure too) with permission from (Alhassan et al., 2015)

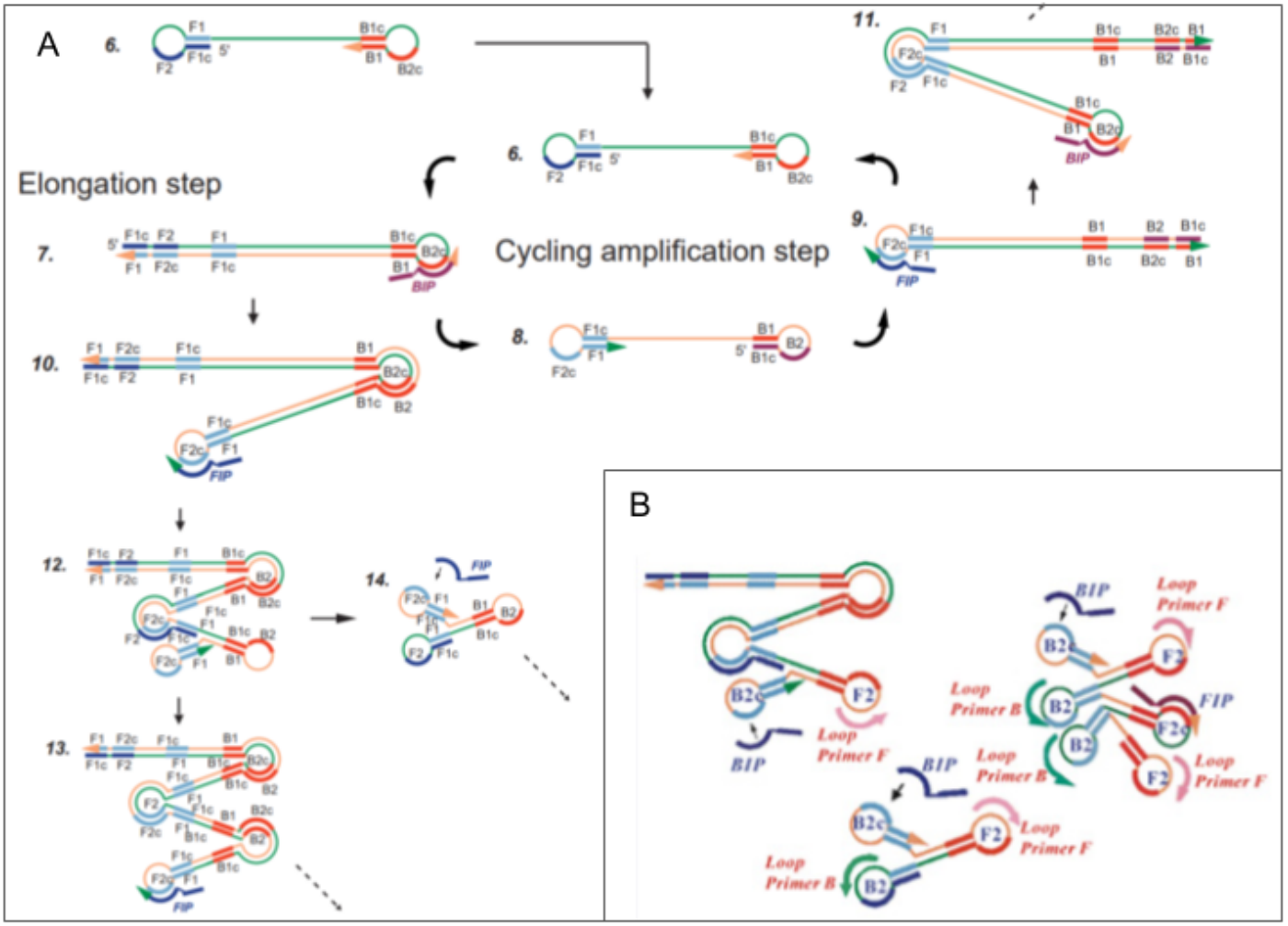

Figure 2.1.8 LAMP Cycling amplification at a constant temperature: A) The dumbbell structures created in the initial reaction (6 and 8) are then elongated through self-priming creating stem-loop structures (7 and 9). Binding/annealing of the FIP/BIP to the single-stranded loop (F2c/B2c) primes strand displacement DNA synthesis releasing the previously synthesized strand. This released single strand forms a new stem-loop structure folding in on itself because of complementarity of F1/B1 and F1c/B1c which is then elongated releasing the previously extended FIP/BIP which now again creates a new dumbbell structure (6 and 8 cycling step) as well as a longer stem loop (10 and 11) which can then be amplified again, creating various sized structures consisting of alternately inverted repeats of the target sequence on the same strand (12-14). B The addition of loop primers accelerates LAMP reactions. The region between F1 and F2 (or B1c and B2c) is hybridized by the loop primer and allows more rapid amplification. For example, in the original method, only two of the six single stranded loops would be used to initiate amplification, with the addition of loop primers all six are utilized. Adapted from EIKEN CHEMICAL CO., LTD Copyright (C) 2005

2.1.3.2 Improvements to LAMP[2]

Over the last two decades, a plethora of improvements have been made to the original LAMP method including, but not limited to, the primers and enzymes used, the protocol as well as the detection methods. A significant advancement was the addition of loop primers (Nagamine et al., 2002) to accelerate the reactions substantially by increasing the number of sites where amplification can occur (**Fig 2.1.8 B**), which can allow LAMP assays to detect targets in less than half an hour. Through the commercialization of LAMP, a number of companies developed improved versions of the polymerase for the reaction; New England Biolabs (NEB) improved the Bst Polymerase through in silico design and evolution to create Bst 2.0 and Bst 3.0 polymerases, with improved amplification speed, yield, salt tolerance, and thermostability. The 3.0 version was designed for increased reverse transcriptase activity such that it can also amplify RNA targets without the need for another enzyme, the 2.0 also comes in a WarmStart® version which allows setting up reactions at room temperature, by disabling the polymerase activity at temperatures under 50 °C (Manual, 2017). Optigene have developed similar enzymes based on the Polymerase of another thermophilic bacteria *Geobacillus stearothermophilus,* which are more expensive (*OptiGene-LAMP-User-Guide-Assay-Design-Primers-1.pdf*, n.d.). And Lucigen have developed LavaLAMP™ which works at higher temperatures (68-74°C), this can allow improved sensitivity, specificity and speed in some conditions ((*LAMP Loop-Mediated Isothermal Amplification Webinar Lucigen*, n.d.)).

Other examples include lowering the likelihood of non-specific amplification and improving sensitivity and specificity by (Wang et al., 2015) through adding dimethyl sulfoxide (DMSO) as well as Touchdown LAMP, where the reaction is started at a higher temperature and then slowly lowered. (Hardinge et al., 2018) were able to detect single copies of their target in some reactions by using purified primers and a preheating step of the template to separate the strands. LAMP reactions have also been adapted for use in a large array of microfluidic type chips (reviewed in (H. Zhang et al., 2019), and paper based devices (Magro et al., 2017; Rodriguez et al., 2016; G. Xu et al., 2016), in addition to being scaled down to nanoliter sized

[2] Some of the improvements detailed here were achieved within the time span of this thesis.

reactions, for so called “digital” amplification which can give more quantitative results (Khorosheva et al., 2016; Lin et al., 2019; Rolando et al., 2018; H. Yuan et al., 2020)

2.1.3.3. LAMP detection methods and targets

A variety of detection methods have been developed for LAMP assays. In general detection methods can be categorized into two groups, end-point detection, where reactions are tested after they are finished to check if amplification has occurred, and real-time, where the amplification is measured as it is happening, which is needed in order to compare reaction kinetics and if some level of quantification is desired (**Fig 2.1.9 A**). Originally, and often still, LAMP amplification products are detected as in PCR by running a portion of the finished reaction in an agarose gel, along with a DNA staining dye. LAMP reactions do not produce single bands but a distinctive “Ladder” like pattern (**Fig 2.1.9 A** left). Other more visual methods have been developed and include; Turbidity derived from magnesium (Y. Mori et al., 2001) or calcium (M. A. Almasi & Aghapour-Ojaghkandi, 2013) pyrophosphate formation which is visible to the naked eye and can be detected with a turbidimeter for real-time measurement. Recently a new system which utilizes the amplified DNA as a trigger for rapid flocculation of suspended particles was described (Mason & Botella, 2019). DNA Dyes such as SYBR-green (Tomita et al., 2008), Hydroxy naphthol blue (HNB) (Goto et al., 2009) and ethidium bromide can also produce a visual change especially under UV light (Amin Almasi, 2012). pH indicators such as phenol red, cresol red, neutral red, m-cresol purple change color with the drop in pH due to LAMP amplification when a minimal buffer is used (Tanner et al., 2015) (**Fig 2.1.9 B**). While other DNA dyes such as SYTO 9 or 82 are appropriate for following real-time LAMP reactions (Oscorbin et al., 2016; Seyrig et al., 2015) (**Fig 2.1.9 A** right). There are two main obstacles with many of these approaches: 1) They usually require opening the tube to add a dye, and as LAMP reactions create a large amount of DNA (10–20 µg ~100x of PCR), some of the amplified products could escape and then contaminate other future reactions (Y. Mori et al., 2001; Yaren et al., 2017) and 2) These detection methods are not sequence-specific, and false positives could be detected. Isothermal amplification methods in general and LAMP in particular, are known for amplifying non-specific products (especially primer-dimers) due to the high primer concentration and processivity of the polymerases (Gadkar et al., 2018; Liu et

al., 2017; Tanner et al., 2012). For a recent review on sequence specific detection methods, including optical, magnetic, piezoelectric, electrochemical and magnetoresistive sensing see: (Becherer et al., 2020), I further address these issues in the results section **2.2.3**.

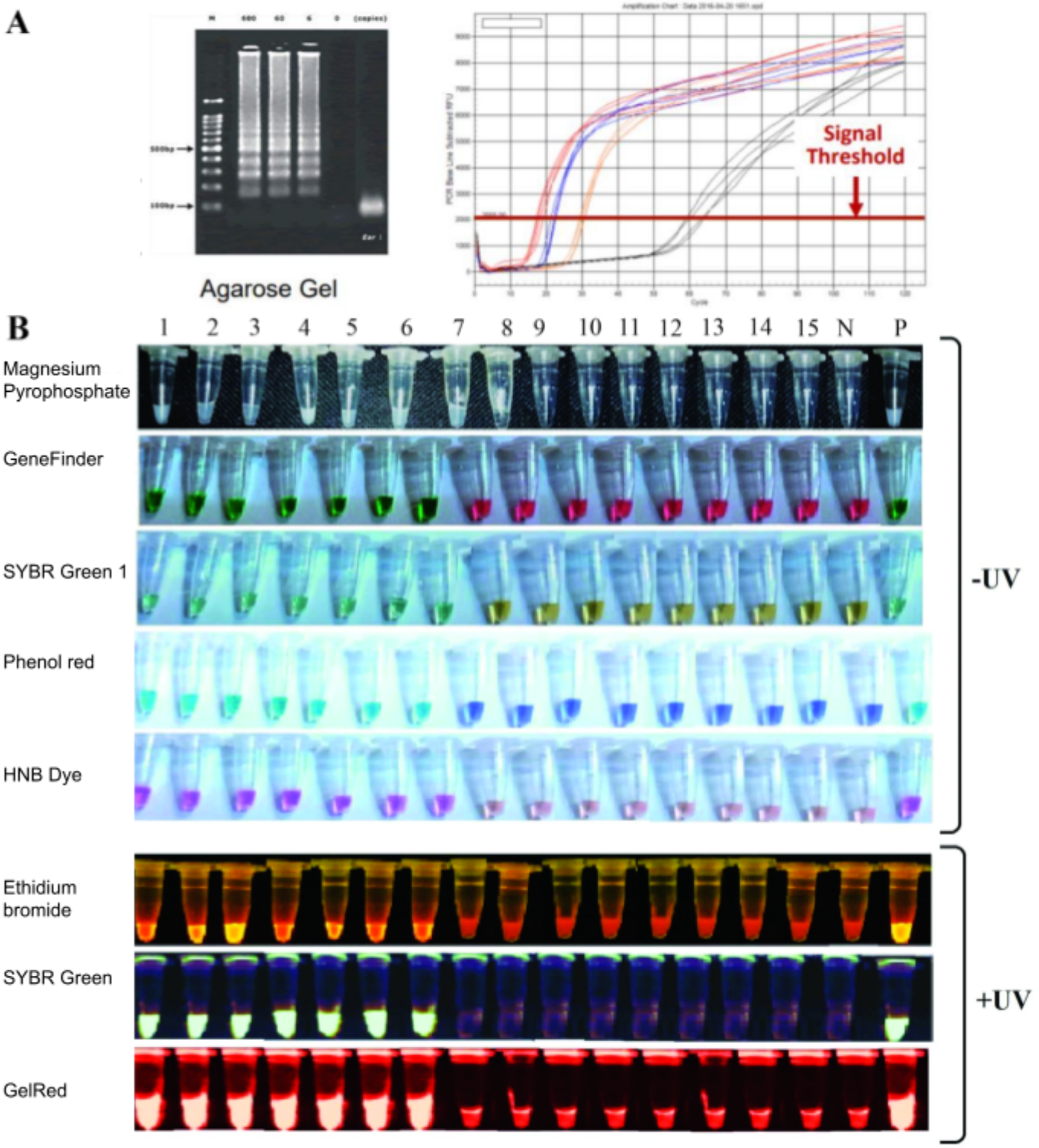

Fig 2.1.9 Different methods to detect amplification of LAMP reactions: A) Endpoint vs real-time detection. LAMP assays run on an agarose gel after the reaction is completed can show if amplification has occurred (left). Following the reactions, as they are happening, allows detection of the kinetics of the reactions but requires more sophisticated equipment (right). **B)** Colorimetric and fluorescent methods allow a binary yes/no distinction to be made by eye, differences of fluorescence are more pronounced but require specific wavelengths. Adapted from (Mohammad Amin Almasi & Almasi, 2017) CC BY-NC 3.0

LAMP is mainly used to detect pathogens and was shown to be sensitive and specific enough to detect RNA and DNA of hundreds of different organisms including viruses, fungi, bacteria and parasites (Dhama et al., 2014a; Kundapur & Nema, 2016; Y.-P. Wong et al., 2018) affecting plants (Aglietti et al., 2019; Rani et al., 2019), animals (Sahoo et al., 2016; Segawa et al., 2014a) and humans (Yasuyoshi Mori & Notomi, 2009; Silva et al., 2019). Some microbes have been detected from biological samples such as blood, serum, urine, cerebrospinal fluid, feces, tissue, and nasal swabs, without the need for DNA/RNA purification (Nie et al., 2012). Nowadays, LAMP reactions for 16 of the 20 neglected tropical diseases recognized by the World health organization (http://www.who.int/neglected_diseases/diseases/en/), have been reported (García-Bernalt Diego et al., 2019), and tests for both malaria and tuberculosis have been commercialized (*Loopamp™ - FIND*, n.d.). Additional commercialized LAMP-based tests exist for a variety of plant pathogens and for the inspection of food (*[EIKEN CHEMICAL] | Molecular Genetics (LAMP)*, n.d., *Plant Health & Pests Diagnostics | OptiGene*, n.d.).
LAMP has been expanding beyond identification of microbial targets (Kundapur & Nema, 2016) such as in the detection of cancer cells, eutrophication, medicinal plants, drug resistance, endangered species, food adulterations, sex determination in humans (Mohammad Amin Almasi & Almasi, 2017) and animals (Centeno-Cuadros et al., 2018; Hirayama et al., 2004; Hsu et al., 2011), allergens (Zahradnik, Martzy, et al., 2014) and other dietary restrictions (M.-S. Lee et al., 2017) and most relevant for us GMOs (Fukuta et al., 2004; Kundapur & Nema, 2016; R. Li et al., 2015).

2.1.4 Detecting GMOs in food and feed

The first commercial genetically modified (GM) plant (the FlavrSavr tomato) was authorized for marketing in 1994, since then, both the amount of different products and the hectarage planted has been steadily growing (James, 2017); In 2015 over 80% of the soybean, almost 70% of the cotton and around 30% of the corn planted worldwide was of a GM variety (Kamle et al., 2017). At the same time, public and governmental attitudes differ around the world, regulation of gene technology varies greatly from country to country, is complex (Marmiroli et al., 2008) and beyond our scope. However, some common issues do exist: assessments on a case-by-case basis, distinctions between contained use, release into the environment and between growing and using (e.g. in food and feed). Tolerance thresholds as a contaminant or for labeling purposes (voluntary or mandatory) exist in some but not all countries and thresholds also vary. This means that the specific needs for detection, identification, and quantification vary significantly. Detecting GMOs may serve several purposes depending on the context and stakeholder. Examples can include legal, safety, or environmental reasons, and can be either qualitative or quantitative (Çakır et al., 2016; Holst-Jensen, 2009). Initially, transgenic crops (soybean, corn, cotton and canola) were developed for agronomic purposes. With few exceptions dominated by herbicide (glyphosate, glufosinate) tolerance (Ht) and insect resistance (various forms of Bacillus thuringiensis (Bt) Cry proteins) (Çakır et al., 2016; Holst-Jensen, 2009). Today the range of transgenic species and their combined traits are being widened.

A typical transgene construct consists of 3 elements; a promoter, which regulates the inserted gene, and promotes the transcription from the DNA, the gene itself, and a terminator that terminates transcription. An insertion of such a construct into a host genome is termed an event. (**Fig 2.1.10 A**) The vast majority of events contain the 35S *Cauliflower mosaic virus* (CaMV) promoter, which was found to express genes strongly in every plant it has been inserted into. In agriculture, over 80 % of GE-crops in the field still carry a version of this element, among those the most widely farmed varieties such as the Roundup Ready soybean, Bt corn and cotton, and the ‘Sunset’ papaya resistant to the papaya ringspot virus(Somssich, 2019).

Another common element found in many constructs and events is the *Agrobacterium tumefaciens* NOS terminator (Tnos) **(Fig 2.1.10 B**). Even though many newer elements have been discovered and can allow more precise control, such as expression in specific tissues, or in response to specific signals, most of the commercially grown strains are so-called “stacked” events where two or more different GE strains are hybridized thus combining the traits of the original strains. (**Fig 2.1.10 C**).

A



B

Common name	Scientific name	Total	35S	Tnos	Neither
Maize	*Zea mays* L.				
Total		46	40	30	1
Single events		27	22	15	1
Stacked products		19	18	15	0
Soy	*Glycine max*	9	6	2	3

C

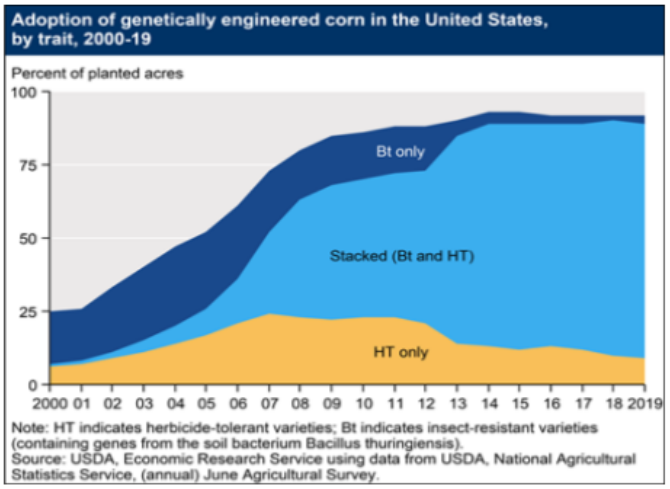

Figure 2.1.10 Common elements in transgenic crops: A) Schematic of a typical transgene construct inserted in a host genome. Arrows indicate the four different assay types targeting: generic transgene sequences (P-35S, T-35S, TNos); sequences within the gene of interest; construct-specific junction sequences; and event-specific junction sequences (Marmiroli et al., 2008) **B**) Table of common genetic elements and their prevalence in commercial strains (Holden et al., 2010). **C**) In the last decade, most planted GM crops are stacked versions, an example of corn in the US (United States Department of Agriculture, Economic Research Service)

GMO detection methods can be broadly categorized into 3 different groups; Phenotypic (Bioassays), Protein (mainly immunological), and Nucleic Acid based, with the last being by far the most commonly used (Morisset et al., 2014; Wilkes et al., 2016). Bioassays here means testing seedlings for a specific phenotype, e.g. resistance to a herbicide. Protein methods are based on looking for a specific protein, usually with antibodies targeting a specific antigen, originally using Enzyme Linked ImmunoSorbent Assays (ELISA), or more recently lateral flow strips (similar to home pregnancy tests). These tests suffer from low sensitivity, especially in processed foods where proteins have often been denatured, but DNA is usually still stable (Elsanhoty et al., 2011; Gryson, 2010; Holst-Jensen, 2009; Pinto et al., 2007).

Nucleic acid based tests can target different regions; Screening tests are based on amplifying a common element that exists in multiple GM constructs, whereas event, construct, or gene specific tests target a more distinct element (Fig 2.1.10 A). Most GMO detection tests use PCR and derivations thereof. Originally screening methods were developed for common elements, tested in collaborative studies and standardized (Bonfini et al., 2012; Lipp et al., 1999). These were mostly qualitative and analyzed by gel electrophoresis. With the growing amount of authorized GM varieties, and regulations, more quantitative methods were developed, mostly based on real-time PCR (Buh Gasparic et al., 2008; Wu et al., 2014; Žel et al., 2011). These methods rely on knowing exactly what you are looking for, which is not always the case. Some newer methods based on high throughput sequencing have been developed, especially in the analysis of new and unauthorized GMOs (Fraiture et al., 2018; Holst-Jensen et al., 2016). (**Fig 2.1.11**). Unfortunately, these methods require expensive, precise equipment and highly trained personnel, which has prompted the development of isothermal based methods for more practical, feasible, low cost, and on-site GMO detection (R. Li et al., 2015; Morisset et al., 2008).

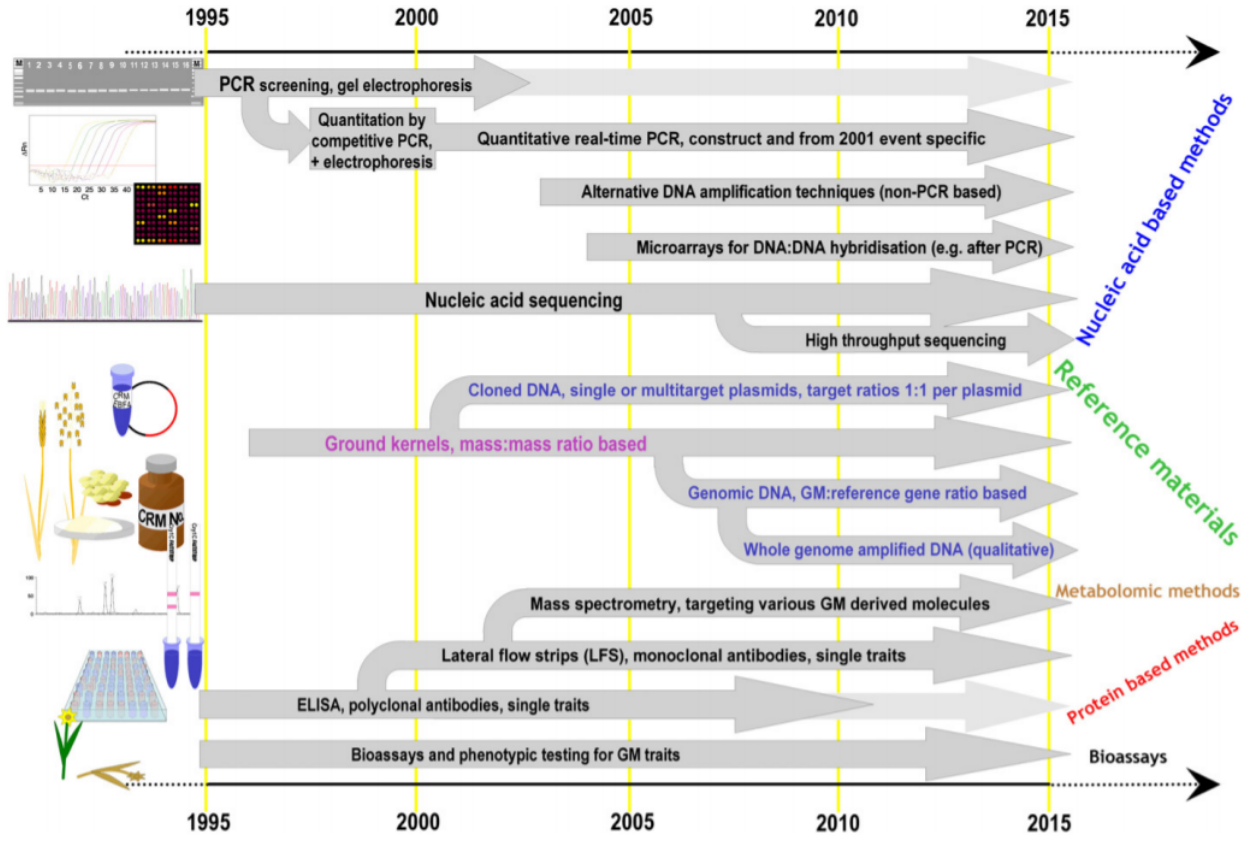

Figure 2.1.11 Evolution of GMO detection methods and associated reference materials. With permission from (Holst-Jensen, 2009)

The first reported isothermal screening method for the detection of GMOs was in 2004, targeting the CaMV-35S promoter and using turbidity for the detection, either by eye or in real-time for semi quantification. The limit of detection (LOD) was reported as 0.5%, as this was the lowest concentration tested (Fukuta et al., 2004). Lee et al. also developed LAMP assays for the 35S promoter as well as for the promoter and terminator for the nopaline synthase gene (P-nos and T-nos, respectively) from *Agrobacterium.* They were additionally the first to report on an event-specific LAMP test for transgenic oilseed rape (*Brassica napus L.*) with LODs of ~8 copies (D. Lee, La Mura, Allnutt, & Powell, 2009). Additional tests have been developed for multiple specific events in corn (Bhoge et al., 2015; L. Chen et al., 2011), soybean (Guan et al., 2010), rice (X. Chen et al., 2012) and cotton (Rostamkhani et al., 2011), as well as for specific herbicide and insecticide transgenes (F. Li et al., 2014; Singh et al., 2015). Recent advances include the reporting of an optimized LAMP assay that allows single copy detection of the 35S promoter and the T-nos terminator in some cases, using **B**ioluminescent **A**ssay in

Real **T**ime (BART) and DaimonDNA: A portable, low-cost loop-mediated isothermal amplification platform for naked-eye detection of genetically modified organisms in resource-limited settings (Hardinge et al., 2018; Kaygusuz et al., 2019).

In the following chapters I will describe how I adapted LAMP GMO detection for use by laypeople.

2.2 Results

2.2.1 Validation of literature primer sets to detect corn, soybean, plant markers, and common transgenic elements

I began by testing LAMP primer sets from the literature that are specific for maize (*Zea mays) (Bektaş, 2017; L. Chen et al., 2011; Kiddle et al., 2012; M. Zhang et al., 2013)* and soybean (*Glycine max*) (M. Zhang et al., 2013), utilizing a new enzyme master mix and dye (WarmStart LAMP 2X Master Mix and Dye, New England Biolabs) in our lab (see methods). Briefly, reactions were prepared according to manufacturer's instructions but scaled down for cost. As template 10ng of genomic DNA (gDNA) was added, extracted according to manufacturer's instructions (DNeasy mericon Food Kit, Qiagen) from 10%w/w or 0% official reference material of transgenic lines of maize (GMO Maize 1507, ERM-BF418D/A) and soybean (GTS 40-3-2, ERM-BF410D/A). Reactions were incubated at 65 °C and tested after 60 minutes. I found that the 10kd primer set detects maize but not water (**Fig 2.2.1. A**). I also found that Soy Lectin primers detect soybean but not water or maize as expected (**Fig 2.2.1. B**).

Next I tested common elements, present in ~85%of planted transgenic crops (Holden et al., 2010; Wu et al., 2014); cauliflower mosaic virus (CaMV) 35S promoter gene (Feng et al., 2015; Fukuta et al., 2004; D. Lee, La Mura, Allnutt, & Powell, 2009; Randhawa et al., 2013; M. Zhang et al., 2013) and the terminator of the agrobacterium tumefaciens Ti plasmid nopaline synthase gene (T*nos*) (Feng et al., 2015; D. Lee, La Mura, Allnutt, & Powell, 2009; M. Zhang et al., 2013). The 35S

primers only detect transgenic maize and soybean but not non-transgenic plants or water (no template control (NTC)) (**Fig 1. C and D**). Similarly, the Tnos primers only detected the modified soybean (**Fig 1. E**) as the GMO maize 1507 we tested does not contain this element (methods)

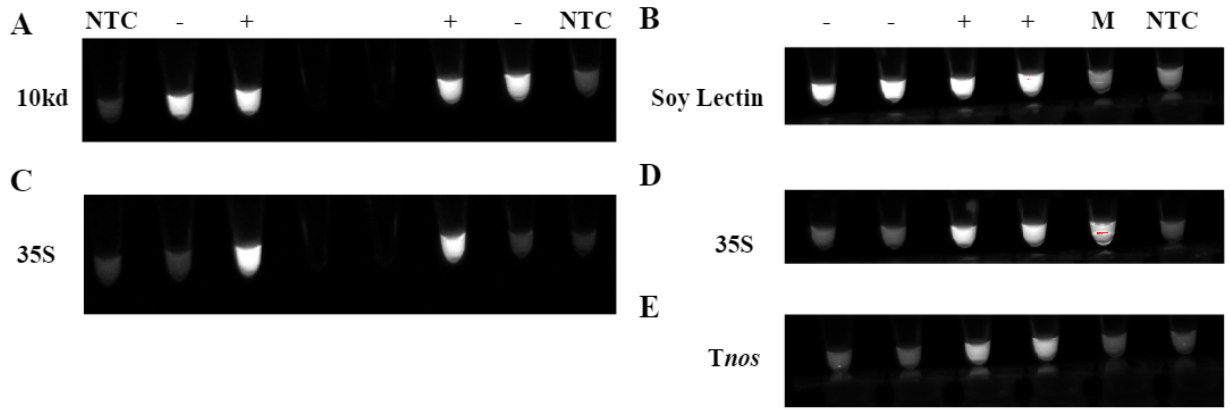

Figure 2.2.1. LAMP primer sets from the literature work in our hands to detect common transgenic elements and corn/soy specific genes. LAMP reactions with a fluorescent dye using primers for: A) Maize 10 kD zein protein, B) Soy Lectin, C and D) Cauliflower mosaic virus 35S (CaMV-35S) promoter gene, E) Agrobacterium tumefaciens Ti plasmid nopaline synthase terminator. +/- , GMO/Non GMO Maize in A and C and GMO/Non-GMO Soybean in B, D and E. M- GMO Maize, NTC, No Template Control(ddH20).

2.2.2 Optimization of reaction temperature

I next optimized and probed the robustness of these reactions to different temperatures when using lower reaction volumes and Bst 2.0 WarmStart polymerase. The speed of the reactions was tested for 8 different temperatures on a gradient between 55 °C and 70 °C. The optimum was found to be between 61 °C and 64 °C. Higher temperatures work as well but take 10-20 minutes longer (**Fig 2.2.3 A**). I wanted to validate this and tested again with a gradient between 67 °C and 59 ° C. The optimum was found to be between 62 °C and 64 °C with a wide tolerance if a slightly longer reaction time is used (**Fig 2.2.3 B**). We can also see in (**Fig 2.2.3 B, 3rd panel and row**) a false-positive result. This is a common occurrence in long LAMP reactions due primarily to primer-dimer amplification (Wang et al., 2015) (methods).

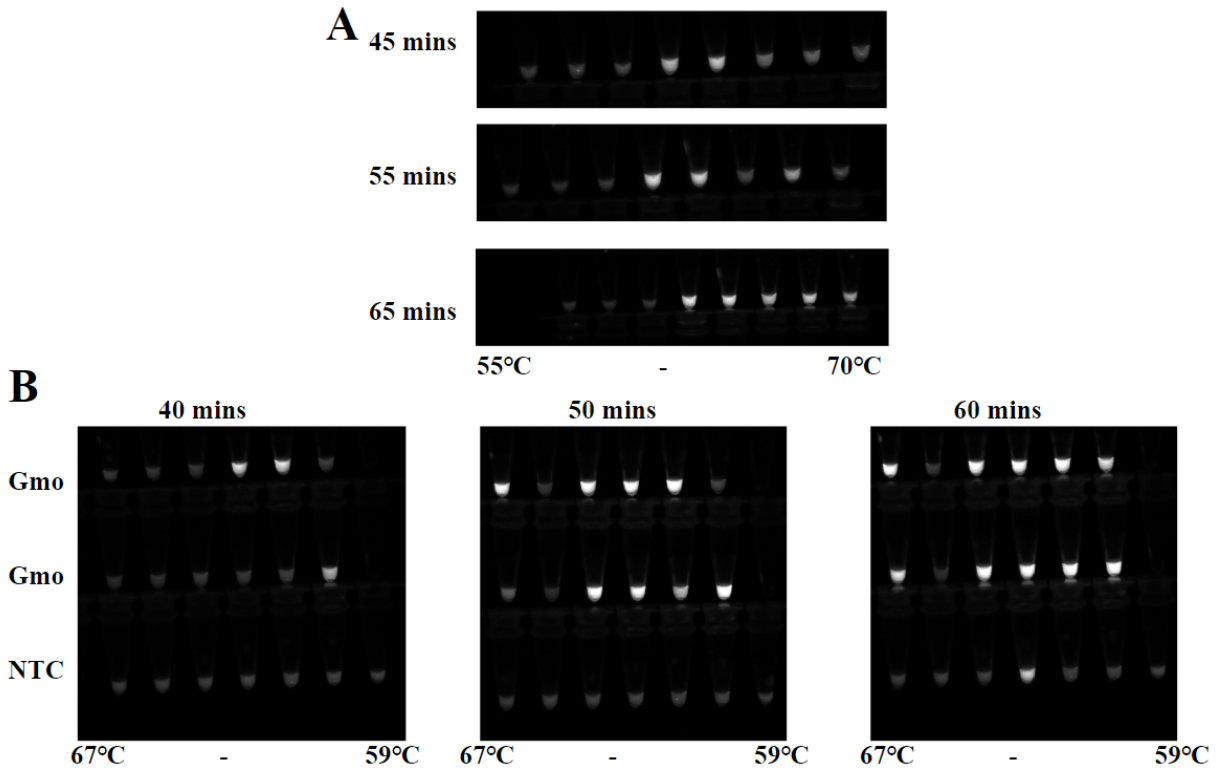

Figure 2.2.3. The optimum temperature for the 35S primer set reaction is ~63°C but is tolerant to a wider range if it is left longer. LAMP results of the 35S promoter set on 10ng of GMO maize on a temperature gradient at different times. A) 56, 57 59 61 64 67 69 and 70 °C after 45, 55 and 65 minute. B) 67/66/65/64/63/61/60/59 °C after 40, 50 and 60 minutes, top row 10% GMO soybean, middle 10% GMO maize , bottom H2O, No Template Control (NTC).

2.3.3 Designing and testing fluorescent, false-positive resistant primers for GMO and plant markers.

While I was working on the Zika Virus two fascinating and timely papers came out (Ball et al., 2016; Priye et al., 2017), which introduced a new detection method for LAMP termed Quenching of Unincorporated Amplification Signal Reporters (QUASR LAMP), which solves the false positive problems, allows multiplexing, and easy detection, without the need to open the tubes. Briefly, a fluorophore is attached to the 5' end of one of the Loop or Inner primers, and an extra short complementary primer is placed in the reaction with a quencher attached to its 3' end. As this primer is short it's Tm is much lower and at ~65 °C it does not bind to its complement, if at the end of a reaction all or most primers are incorporated into double stranded DNA the quenchers cannot bind and the reaction fluoresces, if on the other hand no/little

amplification happened, or only amplification of short primer dimers, then when the tubes are cooled, the quencher will bind to its complementary strand and quench the fluorescence (**Fig 2.2.4**). These differences in fluorescence are large and easy to detect using affordable optics (See section 3.2.3)

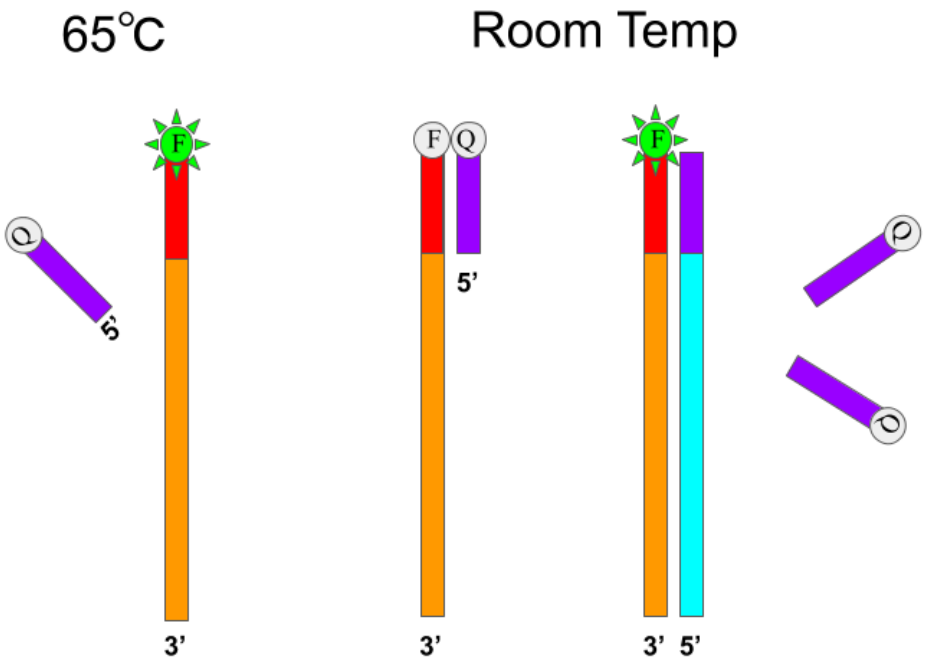

Figure 2.2.4 QUASR Detection: A fluorophore is attached to the 5' end of one of the Loop or Inner primers (Orange), and an extra short (~10bps) complementary primer is placed in the reaction in excess with a quencher attached to its 3' end (Purple). At ~65 °C the short quenching probe does not bind to its complement (left), if at the end of a reaction all or most of the labeled primers are incorporated into double stranded DNA the quenchers cannot bind and the reaction fluoresces (right), otherwise when the tubes are cooled, the quencher will bind to its complementary strand and quench the fluorescence (middle).

I designed and tested QUASR primer sets for our targets specific to maize, CaMV 35S promoter and soybean lectin (**Fig 2.2.5 A and B)**. Using the fluorescein (FAM) dye, on either the Forward or Backward inner primers (FIP/BIP), depending on their susceptibility to forming hairpin structures occupying the 5' ends and self-dimerization or cross-dimerization with other primers in the set using (*OligoAnalyzer Tool - Primer Analysis | IDT*, n.d.) (see also Ball et al., 2016; Priye et al., 2017 and methods). The short quenching primer was attached on its 3' end to a

Iowa Black® FQ quencher. Black Hole Quencher®-1 was also tried and gave very similar results but is slightly more expensive. These successfully detected their targets but not other samples of both official reference standards and derived products. (**Fig 2.2.5 A and B)**

In order to make the simplest kit/experiment I looked for a common gene that could be targeted in all plants as a control and found primers for the cytochrome oxidase (COX) mitochondrial gene common to most plants (Tomlinson et al., 2010). I tested this primer set on maize, soybean and processed food and found it to work well on all samples examined (**Fig 2.2.5 C)**. I also developed and tested a QUASR version of this primer set (**Fig 2.2.5 D)**.

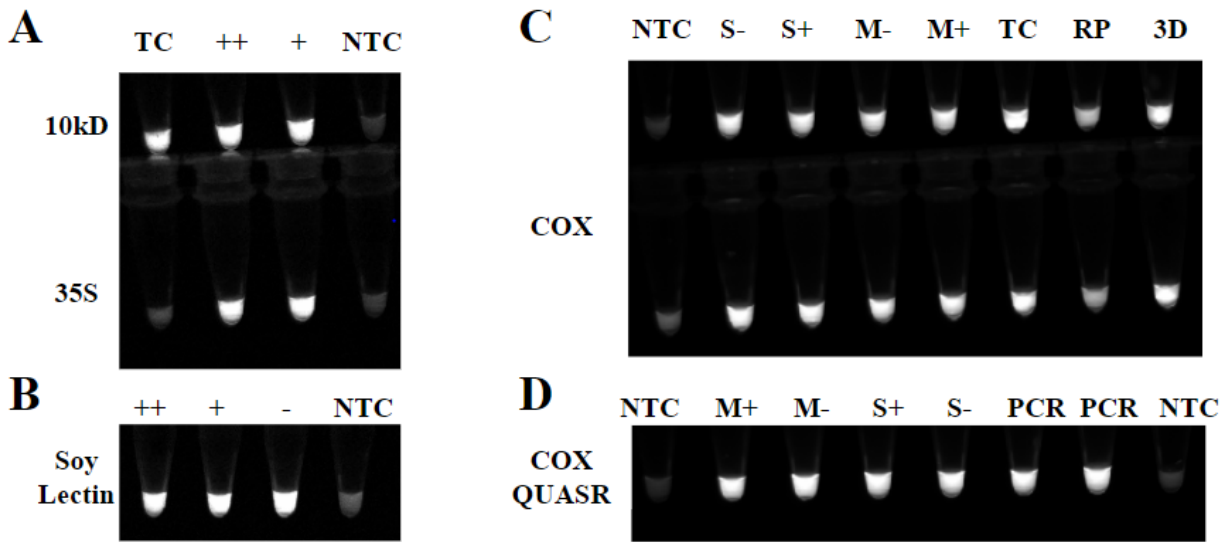

Figure 2.2.5 Fluorescent, false positive resistant primers for GMO and plant markers. A) LAMP results utilizing fluorescent primers and a short quencher primer on TC =Tortilla chips DNA, ++=10ng of GMO maize DNA, +=2 ng of GMO maize DNA, NTC= no template control(ddH2O), with both 10kD and 35S primer sets. B) Same but with ++=10ng of GMO soy DNA, +=2 ng of GMO soy DNA, -=10 ng of non GMO soy DNA, NTC no template control using soy lectin primer set. C) COX1 primer set without fluorescent primers but a non specific fluorescent dye on: NTC no template control, S-=nonGMO soy S+=GMO soy, M-=nonGMO maize M+=GMO maize, TC Tortilla chips, RP Reese's pieces cereal, 3D Bugles. D) LAMP results utilizing fluorescent primers and a short quencher primer on: M-=nonGMO maize M+=GMO maize, S-=nonGMO soy S+=GMO soy, PCR=COX1 PCR product, NTC no template control.

2.2.4 Investigating different fluorophores and multiplexing

As stated, an important advantage of QUASR LAMP is the possibility of multiplexing 2-3 different targets in the same reaction. In order to test this, I attached the HEX and CY3 fluorophores instead of FAM to the FIP/BIP primers, keeping the same quenchers. This successfully allowed detection of different targets (**Fig. 2.2.6 A**). I next asked if the reaction could be multiplex, looking for both a plant marker (as a control for DNA extraction success) and a GMO marker, each fluorescing at different wavelengths in the same tube. These experiments successfully allowed the detection of 2 different markers in the same reaction (**Fig. 2.2.6 B**). I have not continued this avenue of research for two reasons. First, these reactions took on average a longer time to all reach the correct result (~80 minutes and not ~60), this is probably due to the fact that only half the amount of primers usually used for each target was added. This could probably be remedied by increasing/optimizing the primer concentration. Secondly the interpretation and hardware needed to analyze the results are more complex, and as a proof of principle, I first wanted to employ the simplest possible system for lay people.

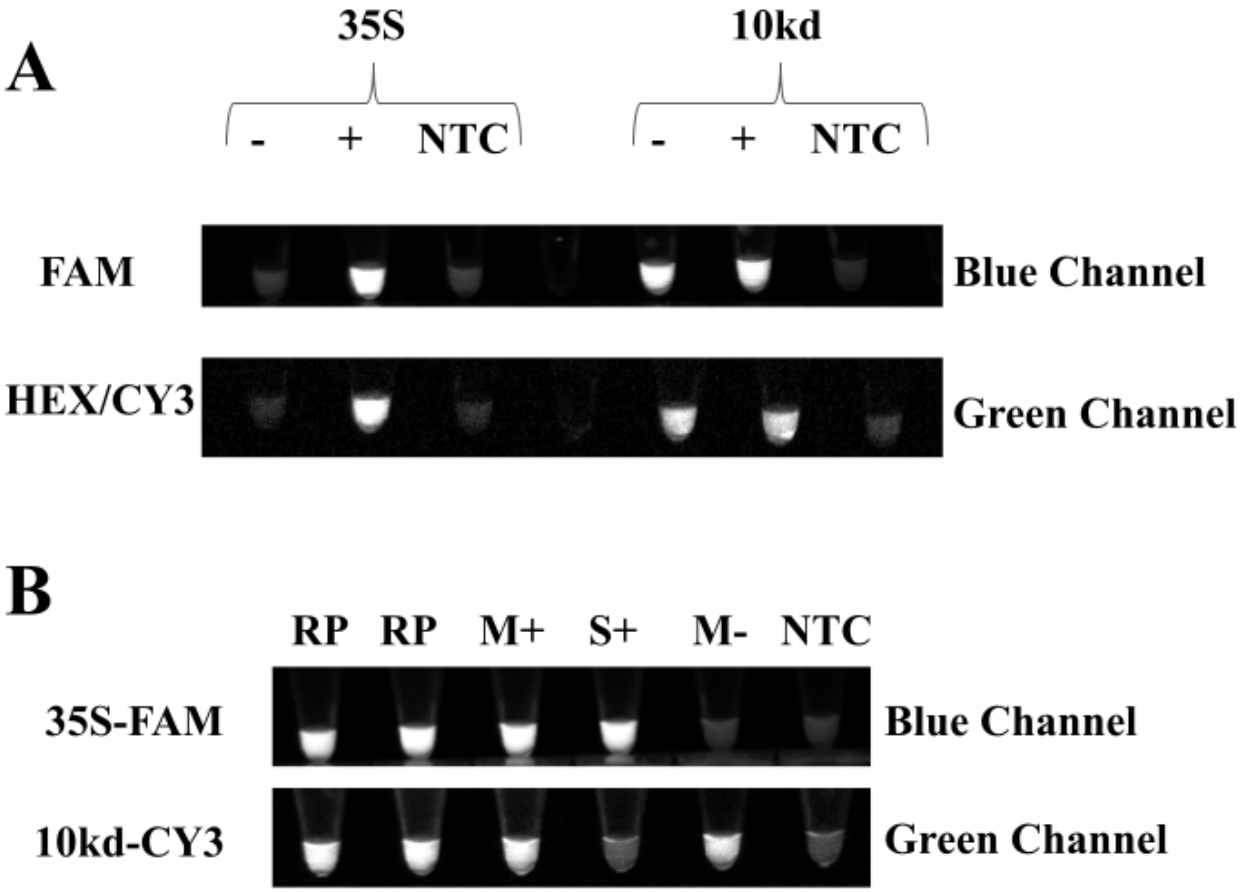

Figure 2.2.6 Using different fluorescent dyes on primers can allow multiplexing. A) LAMP results utilizing fluorescent primers with 3 different dyes, and a short complementary quenching primer. 35S set labeled with FAM above and HEX below and the 10kD set labeled with FAM above and CY3 below. Labels: **-** nonGMO maize, **+** GMO maize, NTC no template control. B) Multiplexed LAMP results after 80 minutes. Same tubes but imaged with two different channels. 35S set labeled with FAM (Blue Channel) and 10kD set labeled with CY3 (Geen Channel). RP Reese's pieces cereal, M+=GMO maize, S+=GMO soybean, M-=nonGMO maize, NTC no template control.

2.2.5 High throughput multiparameter screening experiments

In the vast majority of LAMP papers, if any optimization is reported, usually only the temperature, sometimes the magnesium concentration is changed. Stopping the reactions after different amounts of time, running a gel, or by monitoring the reactions in real-time to measure changes in time to a positive signal. LAMP reactions include multiple components: each of the six different primers (FIP/BIP,F3/B3,LF/LB), the enzyme, salts (magnesium), additives (betaine),

nucleotides (dNTPs), as well as the reaction buffer and DNA sample all of which vary in the reported literature. I sought to understand better which of the different components and their concentrations are pertinent in our situation. Testing for all the different combinations quickly gets out of hand combinatorially, just these 12 components at 2 different levels would take 2^13 (8,192) experiments without repeats or negative controls. Additionally testing 3 levels would be even better as there might be non-linear interactions between the different variables (3^13=1,594,323). A classical one-factor-at-a-time (OFAT) approach to optimization is time and resource intensive, and is not appropriate to yield globally optimum parameters (Czitrom, 1999). Here the field of statistics called design of experiments (DOE) was employed (Anderson & Whitcomb, 2016). DOE efficiently examines multiple parameters in a minimum number of runs (experiments) and has evolved from the work of R.A Fisher in agricultural field experiments (Preece, 1990).

Screening experiments are conducted when there are a large number of potential factors that may influence the response (Anderson & Whitcomb, 2016). The purpose of the experiment was to identify a smaller set of active factors. Traditionally this is done using fractional factorial designs, which are experimental designs consisting of a carefully chosen subset (fraction) of the experimental runs of a full factorial design (where all the different combinations are tested), at two different levels of the variables or categories (Anderson & Whitcomb, 2016). These designs can confound the main effects with two-factor interactions, and cannot capture nonlinear interactions. Recently a new type of screening design has been developed named definitive screening design (DSD) (Jones, 2016; Jones & Nachtsheim, 2011). These three-level screening designs have many desirable properties: orthogonal main effects, which are also completely unaliased (or unconfounded) with two factor interactions. They have a self-foldover structure (each run has another one that mirrors it). Quadratic (non-linear) effects can also be estimated, if their strength is large enough and if certain reasonable sparsity conditions are met. One of the advantages of DSDs, from a practical perspective, is the relatively low number of runs required, which is only roughly twice as many as the number of quantitative factors under optimization (Santos et al., 2019).

I carried out DSD based experiments on our LAMP assays. This was possible using, on the one hand, a powerful nanoliter pipetting robot (Labcyte EchoDrop 550) which can accurately and rapidly transfer 2.5nL droplets using acoustic technology, and a 384 well real-time PCR machine capable of reading the fluorescence of the reactions in real-time in order to know when the reactions are amplifying. Recently Digital LAMP has been demonstrated, at pico to nanoliter volumes (Khorosheva et al., 2016; Rane et al., 2015; Schuler et al., 2016), which suggests that reactions can be further miniaturized from the standard ~10µl reactions I usually do. Indeed my preliminary experiments showed no difference between 3,6,and 9ul reactions when monitored in real-time (data not shown).

I wrote custom MatLab software which takes a DSD design (Albrecht, n.d.) and creates an Echo cherry-pick (*Echo Cherry Pick Software*, n.d.) transfer file (CSV) which can be read by any liquid handler capable of transferring nanoliters from plate to plate. **Table 2.2.1** shows the high medium and low values of the thirteen different variables modulated. A real-time DNA binding dye was added (SYTO 82) with fluorescence in a different range (orange) than the FAM tagged QUASR primers (green). Experiments were done in quadruplicate, at 3ul total volume in a 384 real-time PCR plate at 63 °C for 90 minutes followed by heating to 95°C for melting curve analysis and then cooling to room temperature for QUASR reading, with fluorescence measurements every minute monitoring both wavelengths. Negative controls were also run in quadruplicate, with the sample level corresponding to different purities of water or fruit fly DNA. DSD design (runs/experiments) and time to positive are shown in Appendix A (also see methods for more details).

	High(+1)	Medium(0)	Low(-1)
Buffer (X)	1.5	1	0.5
Bst Enzyme (U/µL)	0.32	0.16	0.8
MgSO4 (mM)	10	8	6
dNTPs (mL)	1.4	0.9	0.4
Betaine (M)	0.9	0.45	0
Primer FIP(mM)	1.6	0.8	0.4
Primer BIP(mM)	1.6	0.8	0.4
Primer F3(mM)	0.2	0.1	0
Primer B3(mM)	0.2	0.1	0
Primer FLP(mM)	0.8	0.4	0
Primer BLP(mM)	0.8	0.4	0
Sample+(ng/ul)	5 Soy	5 Corn	2 Soy
Sample-(ng/ul)	5 Fly DNA	0 ddH2O	0 Sink H20

Table 2.2.1 Values of the different components at high (+1) medium (0) and low (-1) settings

These screening experiments revealed the important factors affecting the speed of the reaction. These include, by order of importance, the sample DNA concentration, the concentration of dNTPS, and the concentrations of both the forward and backward loop primers (**Fig 2.2.7 A and B**). Interestingly the other factors had very little influence on the time to a positive result. A model taking only these four factors into account is able to predict the data reasonably well (R^2=0.55) (**Fig 2.2.7 C**) (methods). This moderate correlation could be explained by my choice of parameters. Although all based on the literature, these were set more towards the lower end, in an attempt to lower further the costs of the reactions in general. This can be seen in (**Fig 2.2.7 C**) as ~half of the reactions failed to amplify. Despite this, the low, medium and high settings of all factors were present in the subset of reactions that did amplify (Appendix A). The surprising result of a lack of correlation between the amount of enzyme as well as the relatively long FIP/BIP labeled primers to the time to a positive amplification, did allow us to further optimize the assay in terms of cost, lowering the price a further 25% (See next Chapter section 3.2.6).

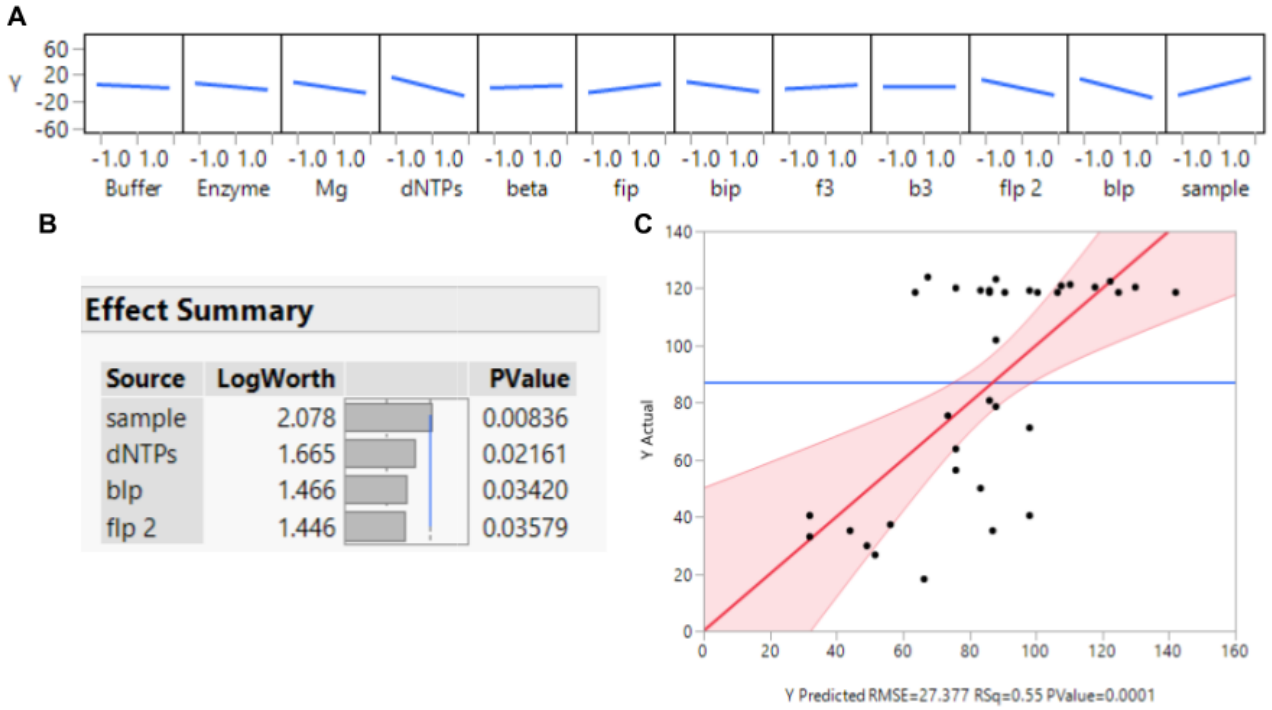

Figure 2.2.7 Definitive screening design allows determination of the most important factors influencing the time to a positive amplification result. **A**) Correlations between the low/medium/high (-1/0/1) levels of the different parameters (reaction components) vs Y - normalized time to positive result). **B**) Significant terms affecting time to positive, sorted by their P-Value, and LogWorth for each model effect, defined as -log10(p-value). **C**) Predicted vs. Actual time to positive result, based on a model taking only the 4 previous parameters into account.

2.2.5 Robustness

While usually, LAMP reactions are only optimized for speed, sensitivity and specificity, I was also especially interested in robustness. I saw that the most difficult part of doing molecular biology for lay people is precise pipetting (see next chapter for more details). I wanted to ensure that reactions are also **robust** to pipetting mistakes of the key factors, namely buffer and sample, as these are what need to be added to a freeze-dried, shelf-stable reaction (see section 3.2.2). Both COX and 35S primer sets were tested along with either 1% w/w GMO soybean, 10% w/w GMO maize, and water, using the newly optimized reactions (lowering the enzyme and FIP/BIP, while raising the dNTPs and FLP/BLP concentrations) (methods). Here total reaction volume was not kept steady. Adding either half or 1.5x of the sample did not

significantly affect time to a positive result (**Fig 2.2.8**). Similarly adding either half or 1.5x of the isothermal amplification buffer still resulted in comparable times. Surprisingly lowering the amount of buffer actually resulted in better performance, this might be due to a more optimized concentration of salts, or pH and could be further investigated. Lowering the concentration of the enzyme by half from the already lowered concentration did slow down amplification of the 35S reactions significantly, but not for the COX primer set, this is probably due to the much higher concentration of the COX gene in a sample, as well as the shorter DNA length of mitochondrial DNA. LAMP has been shown to be robust to changes in pH and temperature (Francois et al., 2011), here I show it can also be robust to pipetting errors.

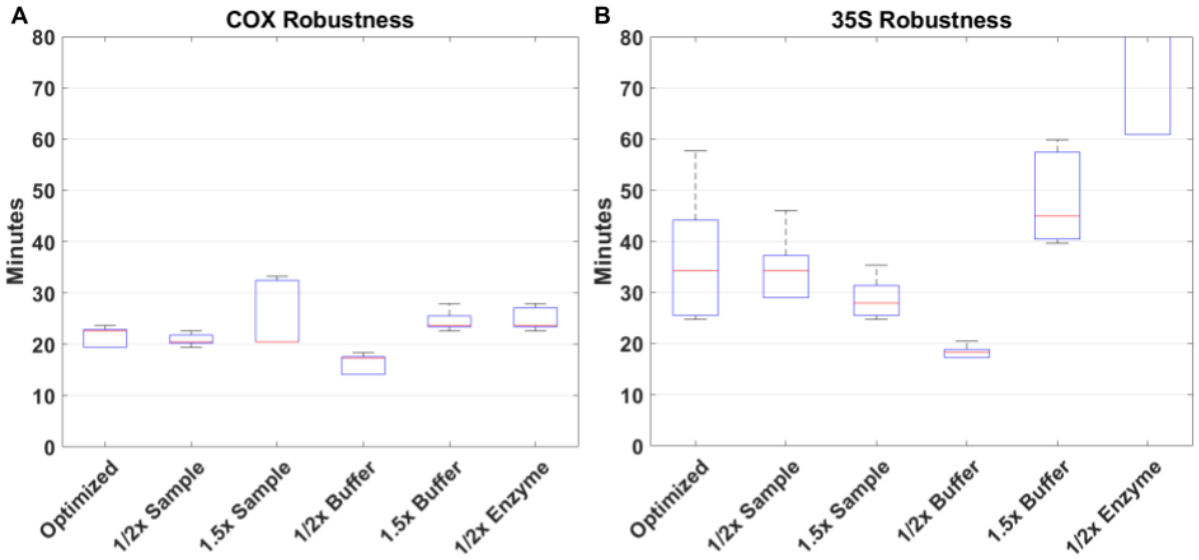

Figure 2.2.8 Newly optimized reactions are robust to pipetting errors. LAMP reaction time to positive (measured using a LC480 qPCR machine) of either **A**) COX or **B**) 35S primer sets with normal, half or 1.5x of either sample or buffer. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points. Triplicates of either 1% w/w GMO soybean, 10% w/w GMO maize are aggregated.

2.3 Discussion

Although detection of GMOs through loop mediated amplification was demonstrated 15 years ago (Fukuta et al., 2004), it has still not been widely adopted. By making it

easier to use, affordable, and robust we were able to use this assay with hundreds of laypeople around the world, as an educational/citizen science tool. I first adapted published LAMP primer sets and reactions for use with a WarmStart® (NEB) enzyme which allows set-up of the reaction at room temperature, removing the need for ice. Reactions were also scaled down for cost. Additionally, primers were adapted so the reactions would be more resistant to the common problem of false positives, and that amplification could be detected easily through fluorescence with simple optics, adopting the newly proposed QUASR method. This could also allow multiplexing in the future. Using new high throughput methods I was able to optimize the reactions for not only sensitivity and specificity as is usually done but also for affordability and robustness to pipetting errors. The next chapter will illustrate how I innovated in regards to the sample preparation, hardware, and protocol in order to allow this to be as easy as possible and to scale.

Here I focused on detecting the most common element in GMOs, namely the 35S CaMV promoter. But as discussed GMOs do exist without this element, and in the future, as new products are developed there will be more of these. Similar work could be done in order to detect other common elements or specific genes, events or constructs depending on the need and people's interest. Primer sets for some of these have already been published and others could be developed. For example, a test specifically for glyphosate/roundup resistance. Or for the first genetically engineered animal recently approved for human consumption in the United States and Canada, the AquAdvantage salmon (Office of the Commissioner, 2019; Yaskowiak et al., 2006), which grows more rapidly and to larger size through the insertion of a modified growth hormone. A group of citizen scientists in Seattle is already working on detecting different types of salmon using LAMP (*CitizenSalmon*, n.d.). If indeed more targets were being sought out, multiplexing might become more useful. This would require further optimization of primer sets and their concentration, in addition to more complex detection and interpretation.

This work is to my knowledge, the first time where a multi-factorial methodology (design of experimentsl) was applied (i.e. not one factor at a time) to gain insight and optimize a LAMP assay. The only related work I have seen is a poster in an industrial

setting improving a luminescence based enzymatic assay using definitive screening (Vidugiris et al., n.d.). Other examples in the biological field include, optimization of mass spectroscopy parameters (Hecht et al., 2015), and efficient characterization of an E. coli fermentation process for recombinant protein production (Tai et al., 2015). These methodologies could substantially improve LAMP assay development in a variety of contexts, but do require sophisticated planning, liquid handling robots and data analysis. New software is being developed to help with these (Nematollahi et al., n.d.). My screening could have been improved with a more careful selection of the parameters. Other screening techniques such as fractional factorial, central composite or box-behnken designs could be tried alternatively (Santos et al., 2019). Additional optimization might be achieved by modifying the reaction buffer. Here I simply used the isothermal amplification buffer which is recommended by the enzyme manufacturer, but preliminary results show this might be improved. Finally, further insights might be gleaned from running LAMP reactions in a digital format i.e in droplet or microwell format, where on average one or no amplifiable molecule is present. This could allow differentiation between two different scenarios when an improved time to positive is observed 1) a larger fraction of templates amplifying (improved ‘fates’), efficiency or sensitivity or 2) a change in reaction rate or speed (Khorosheva et al., 2016; Rolando et al., 2018).

The work described in this chapter was all done in laboratory conditions using clean highly purified DNA and expensive precise machinery. The next chapter will discuss how I was able to make this all much more accessible, taking it out of the lab and into the hands of students and the general public.

Chapter 3:

Lowering barriers to DNA detection

3.1 Introduction

3.1.1 Technical Hurdles

A major aim of this work was about taking molecular biology outside of the lab and getting it into the hands of laypeople and students in the real world, to allow them to detect a sequence of interest easily, rapidly and affordably. Currently, for historic and economic reasons molecular biology research and industry is mostly concentrated on tools for well-equipped labs, which assumes the use of specialized, complex and expensive equipment, which limits who can participate (Damase et al., 2015; Whitesides, 2011). Recent attempts to make molecular biology tools more mobile, affordable and accessible such as the Bento Lab mobile genomics setup (*Bento Lab - Experience Genetics Anywhere*, n.d.), Franklin™ by Biomeme Mobile qPCR Thermocycler (https://biomeme.com/), the Feles Desktop Biolab (https://www.felesbio.com/), or the educational amino DNA Playground™ (https://amino.bio/) still cost between 500$ and 5000$ which is prohibitive in many contexts, and suffer from low throughput so that multiple machines would be needed in a class or workshop environment. To reach our goals required overcoming a variety of technical hurdles, as well as innovating in regards to the hardware and protocol.

Successfully conducting a LAMP experiment requires a few different steps: Taking samples and extracting the DNA, then adding this DNA to a reaction mixture containing the enzyme along with primers nucleotides and buffer, followed by isothermal heating and then analysis of the results. The following introduction describes how these are usually done in the lab, as well as newer solutions which can help to make DNA detection more accessible.

3.1.2 DNA extraction

As previously stated (sections 2.1.2 and 2.1.3), LAMP reactions are less sensitive to inhibitors than traditional PCR, but still require accessible DNA in order to successfully amplify their target sequences. Many scientists still use variations on classic organic protocols for extracting DNA from food and plants. These include adapted versions of the Cetyl TrimethylAmmonium Bromide (CTAB) method (Doyle & Doyle, 1987; Inglis et al., 2018; Murray & Thompson, 1980; Tamari & Hinkley, 2016) or SDS-potassium-acetate method (Dellaporta et al., 1983; Hosaka, 2004) which are relatively complex, expensive, time-consuming and require a full lab, highly trained personnel, in addition to the use of potentially hazardous chemicals. For example in the lab we commonly use a commercialized version of the CTAB method (Qiagen food or plant kits) for DNA extraction, that needs quantification and/or other downstream applications (**See Fig 3.1.1 A**) First a homogenizing step, followed by a lysis step using proteinase K, a lysis buffer and heating on a shaking thermal block/bath for 30 minutes at 60°C. The next step is centrifugation at 2500 x g for 5 mins and then taking the supernatant and adding it to chloroform in a chemical hood. This is followed by vortexing and spinning at 14,000 x g for 15mins. Then taking one phase into another buffer vortexing again and passing the mixture through a silica column and centrifuging twice at 17,900 x g and then eluting into a new tube. This whole process takes at least an hour and requires equipment costing thousands of dollars, dangerous chemicals and careful manipulation, not ideal for our intentions.

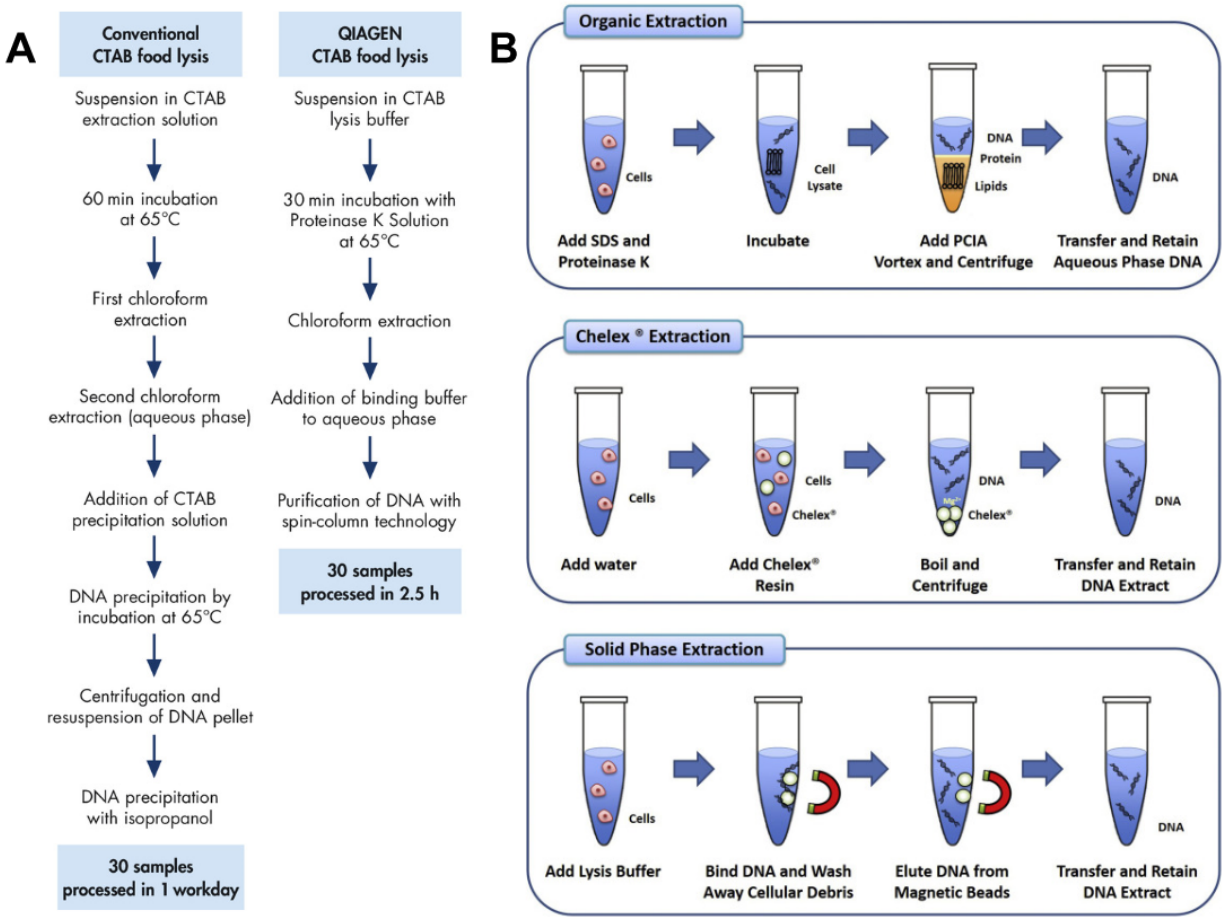

Figure 3.1.1 Common DNA extraction procedures A) Comparison of conventional CTAB DNA extraction method with the shorter Qiagen© DNeasy Mericon Food Kit (*DNeasy Mericon Food Kit - QIAGEN Online Shop*, n.d.)
B) Illustration comparing three major methods of DNA extraction. Top: Organic extraction using phenol:chloroform:isoamyl alcohol (PCIA) partitions DNA to the aqueous phase while lipids and proteins are partitioned to the organic phase or the aqueous–organic interface. Middle: Extraction by chelating resin (e.g., Chelex®) releases DNA by boiling and inactivates degradative enzymes by chelating essential divalent cations such as Mg2+. Bottom: Solid phase extraction selectively binds DNA to a solid support such as silica in the presence of high salt solutions. By coupling silica to paramagnetic particles, magnets can be used to immobilize DNA while washing away other cellular components. Adapted from: (McKiernan & Danielson, 2017)

Other simpler DNA extraction protocols have been proposed throughout the years. Commonly used and newer techniques for DNA extraction include 1) Resin based extraction such as Chelex 100® (Walsh et al., 2013) and Silica (dnabarcoding101.org, n.d.), which can selectively bind DNA or ions under the right

conditions 2) Solid phase extraction where DNA is selectively bound to a solid support such as silica or magnetic beads in the presence of high salt solution, then washing away the debris, and eluting (**Fig 3.1.1 B**). These methods still usually require a centrifuge and multiple pipetting steps and tubes (S. C. Tan & Yiap, 2009) (see figure) in addition to being quite expensive(~1-5 per extraction), and adapted for a lab setting rather than for the field or for lay people.

A rapid single-tube method for DNA extraction called alkali boiling also appears in the literature (Bektaş, 2017; Klimyuk et al., 1993; Yahya et al., 2017; W. Zhang et al., 2014), where cells or foods are boiled at a high pH (>13), using a 0.1-0.5M NaOH solution at 95-100 °C for 30 seconds to ~15 minutes and then neutralized or not. This method has the advantages of requiring few steps and equipment, thus making it simpler and less likely for cross-contamination to happen, but does use a caustic solution. I tested a variety of these methods for their appropriateness in our context. Lastly, a variety of direct PCR or LAMP amplification methods have been proposed, where the starting material is barely to not at all processed before amplification. Berthomieu & Meyer (1991) and Levin & Gilboa (1997) added tomato pollen grains or cabbage and tobacco (leaves and roots) directly to PCR reactions successfully. Sharma et al. (2012) Tested a similar method on a variety of plants, using alcohol desiccated leaves. Ben-Amar et al. (2017) suggested direct PCR from a variety of samples by first grinding them in TE buffer then heating and mixing for 10 minutes followed by centrifugation. D. Lee, La Mura, Allnutt, Powell, et al. (2009) showed direct LAMP amplification after simply grinding maize grains and leaves in water, and Tsai et al. (2016) showed sex determination using LAMP directly from leaf discs of the papaya plant. Here (see section 3.2.1), I tested and refined the simplest and fastest of these methods and developed a novel and robust 6 minute DNA extraction protocol that only required water.

3.1.3 Wetware/Reagents

From my own experience, if you are not an established lab from academia or industry in the global north, getting enzymes is complex, costly and can take weeks

to months. When I tried to get a common enzyme (NEB Bst2.0 polymerase) delivered to a public university in Brazil, it took almost 2 months, cost 5 times more than it would in the global north, and when it arrived it was warm (the cold chain was not kept) and did not work. Similar complaints and issues have been raised by many colleagues working in Latin America (Cruz et al., 2016), Africa (*Capacity Building for the Bioeconomy in Africa Bakubung Workshop Report*, n.d.) and Southeast Asia (Dean et al., 2018). One group trying to address this issue is the Open Bioeconomy Lab (https://openbioeconomy.org) "I learned that in many parts of the world, reagents are not affordable and take a long time to arrive... We plan to create an open-source toolkit for the production of enzymes... for people working in low-resource laboratories. The toolkit will consist of hardware designs, the DNA required to make E. coli bacteria produce the enzymes of interest, protocols, and quality-control guidance. It will be as readily available as possible and we are co-developing and piloting it with colleagues in Ghana and Cameroon." (*Biomakespace Projects: Enzyme Toolkit to Make Molecular Biology More Accessible. :: Biomakespace*, n.d.) Indeed I have recently received plasmids from them and FreeGenes that include off patent polymerases capable of carrying out LAMP reactions.

A similar problem also arises when primers or synthetic DNA is needed. And again what would take days in the global north usually takes weeks to months to arrive in the global south (personal experience in Brazil and personal communication with colleagues in Sri-Lanka and Chile). Specifically for QUASR LAMP, this can be an even more acute problem as these reactions require 7 different primers for each target, 2 of which are relatively long(40-50bps) and 2 of which need further modifications (a fluorophore and a quencher, which also requires expensive and complex HPLC purification; see section 2.3.3). Acquiring a positive DNA control for the target we are looking for is also not straightforward everywhere. In our lab we use the European Commission's Joint Research Centre (JRC) certified reference material (CRM) which costs about 60$ per gram of maize or soybean with a certified amount of a specific GMO strain mixed with a non-GMO strain. Ordering a synthetic fragment of the whole amplified sequence (~300bp) or a plasmid containing it would also cost at least 90$ (IDT gBlocks) and would then need to be amplified. I address

these issues of wetware availability by lyophilizing (freeze-drying) the enzyme, primers, nucleotides (dNTPs) and appropriate controls such that they can last when stored at room temperature and all that needs to be added is a room temperature stable buffer along with sample DNA and/or water.

Lyophilization is the drying of materials in a frozen state by sublimation i.e the transfer of solid water straight to gas by sufficiently lowering the temperature and pressure. It has been known for decades that lyophilization of biological materials can keep some heat-sensitive samples stable for years (Everse & Stolzenbach, 1971). It was also found that adding the sugar trehalose can help stabilize enzymes for long periods of time and at different temperatures (Colaço et al., 1992). It is ever more common to lyophilize ready mixes of PCR and qPCR especially for use in the field (*In Pursuit of a Shelf-Stable qPCR Mix*, n.d.) and in low resource settings where keeping a cold chain can be a problem. This method has also recently been shown to work for a few different LAMP-based assays, for the detection of disease-causing agents (Carter et al., 2017; García-Bernalt Diego et al., 2019).

3.1.4 Isothermal Heating

After adding the DNA to the LAMP reaction, isothermal heating of the tubes is needed for 20-90 minutes at 60-75℃ depending on the exact reaction conditions. In a lab, this is usually done using a conventional PCR thermocycler, or a dry/wet heat bath (If using a dry bath, its lid must be heated to avoid evaporation, otherwise oil needs to be added on top of the reaction). These are all quite expensive (300-10000) and not especially portable. Newly developed commercially available devices for isothermal amplification include the Optigene© Genie II or III, which can perform real time LAMP, monitoring the fluorescence levels in the reactions as they happen, which allows some quantification as well as melting curve analysis to safeguard against false positives. These devices cost 3000-15000 and are only capable of doing 8 or 16 reactions at a time. A similar device developed by ©Biomeme called the Franklin™ is geared towards doing real-time PCR in the field, it can read 9 tubes at a time and costs 6000-10000. Open or mini PCR machines can also work but these cost between 400-1000 and can also do only 8 reactions at a time. (Priye et al., 2017) developed a LAMP box to detect arboviruses using a

smartphone, which uses resistors and an aluminum plate as a heat source, although practical, this device still costs ~100$ and can also only do 8 reactions simultaneously and needed specialized reaction vessels to work well. (Liao et al., 2016; Song et al., 2016) Created a portable “Smart cup” which does does LAMP reactions on a microfluidic chip, with heating accomplished by coupling an exothermic chemical reaction (using a disposable commercially available Mg–Fe alloy hand-warming pouch ~$0.15 per pouch) to a phase change material (PCM) with a melting temperature of 68 ◦C to keep the reactions at a relatively constant temperature for an hour. Four different reactions could be done in parallel on their chip and no cost was given for the entire setup. (Velders et al., 2018) recently developed an open-hardware, open-source, Arduino based shield for LAMP which used polydimethylsiloxane (PDMS) as material for the heating block and a nichrome wire for the heating. The cost of the components for building an Arduino LAMP shield, thus without the Arduino board and the batteries was less than 1.5 € when purchasing the parts from Chinese retailers. This was for a setup which could only heat one tube, but this could easily be scaled up in theory. I experimented with a variety of methods and found an affordable and easy way to heat up to hundreds of reactions at the same time for a workshop or class setting.

3.1.5 Analysis and detection of amplification

After isothermal heating, the results of the amplification need to be analyzed. As discussed in the previous chapter, I chose to use Quenching of Unincorporated Amplification Signal Reporters (QUASR LAMP section 2.3.3), mainly as it is one of the only sequence-specific yet relatively simple, rapid and affordable detection methods (Becherer et al., 2020). This requires detecting a fluorescent signal. In the lab, this is commonly achieved using expensive equipment with precise optical filters and light sources, such as the ChemiDoc™ (Bio-Rad) gel reader, or fluorospectrophotometer (e.g. plate readers). Some portable solutions previously mentioned include the Optigene© Genie II or III, and the ©Biomeme Franklin™, but these are still cost-prohibitive. The minPCRs blueBox™ S Transilluminator with Imaging Hood could do the job and costs only 250$. Recently the educational cell-free expression system called BioBits (Stark et al., 2018) showed a design of an 8 tube reader which costs only 15$ to build. A similar design is now commercially

available from minPCR called the P51™ Molecular Fluorescence Viewer for ~25$. Here, I developed a similar, easy to build and fix open-source version before we were aware of their design, which costs only ~1-2$.

The results section (3.2) will show how we were able to make every step in LAMP detection for GMOs more simple, rapid and affordable. Then how we packaged these innovations and strategies into an open modular workshop, such that GMO detection could be taken out of the lab and placed into the hands of the general public and students in any room, only requiring an electrical socket.

3.2 Results

3.2.1 Testing of novel, simple, rapid DNA extraction protocols for LAMP GMO detection

Most nucleic acid-based techniques require complex, long and expensive extraction procedures, utilizing solvents and centrifuges which are inappropriate for our purposes. LAMP has been shown to be more robust to “dirty” samples (than PCR) (Chander et al., 2014; Francois et al., 2011). I developed, adapted and tested, novel simple rapid DNA extraction protocols of maize, soybean and processed foods which contain them (methods). Rapid extraction protocols can be broadly categorized into two - those based on water and those based on alkaline boiling. I adapted the protocol reported by Lee et al. (D. Lee, La Mura, Allnutt, Powell, et al., 2009), which grinds the sample in water (**Fig 2. A, tubes 1 and 6**), but this did not work in our hands. I also scaled down the volume and mass (by 100) and tested the alkaline boiling method reported by (Bektaş, 2017) on grains of corn. This method involved grinding the dry sample then adding 0.1M NaOH and heating for 17 minutes at 95 °C (**Fig 3.2.1 A, tubes 2 and 7**). Another alkaline boiling method was adapted from (W. Zhang et al., 2014) where 0.25M NaOH was added to the ground sample and boiled for 30 seconds then TE buffer was added to neutralize the solution, followed by heating again for ~5 minutes (**Fig 3.2.1 A, tubes 4 and 9**). I also tested a simpler

alkaline boiling method, in which 0.1M NaOH was added to the ground sample and then boiled for 5-10 minutes (**Fig 3.2.1 A, tubes 3 and 8**). Finally, I also tested grinding the sample (0.005g Maize reference material) using a polypropylene pestle made for microtubes (Axygen) then adding 60µL water and boiling for 5 minutes. Boiling serves two purposes, first breaking open cells and secondly denaturing enzymes that might interfere with the following reactions, such as DNases (**Fig 3.2.1 A, tubes 5 and 10**). All 4 mentioned methods allowed successful detection of the Maize 10kd gene when a 2µL sample was used as a template for a 10µL LAMP reaction (**Fig 3.2.1 A)**.

As I was interested in the simplest and most rapid protocol, which could be done with the fewest steps and equipment, I chose to continue to test and optimize the water boiling and simplest alkaline boiling methods. I tested how sensitive the protocols are to the temperature of the boiling step. Water (**Fig 3.2.1 B columns 1-3**) or 0.1M NaOH (**Fig 3.2.1 B columns 4-5**) was added to ground samples which were then kept for 5 minutes in a beaker of 1) boiling water 100 °C (**Fig 3.2.1 B column 1**), 2) Water that starts boiling but cools down slowly due to being on a USB hot plate to 90 °C (**Fig 3.2.1 B columns 2 and 4**) and 3) Water that starts boiling but cools down naturally to around 80 °C (**Fig 3.2.1 B columns 3 and 5**). I found that for both with water and NaOH, the method where the samples are simply placed into boiling water that is allowed to cool naturally (100- 80 °C) was successful in detecting the maize 10kd gene when 2µL sample was used as a template for a 10µL LAMP reaction (**Fig 3.2.1 B columns 3 and 5**).

I wanted to test these methods not only on standard reference maize as in **Fig 3.2.1 A and B**, but also on common food samples, with corn as the main ingredient. I purchased at a local supermarket: Tortilla chips (Pocoloco, Belgium), 3D Bugles (Pepsico, Belgium) and Cheese puffs (Crackets croustillants, goût fromage, Monoprix, France) and ground either 0.005g or 0.01g to which I added either DNAse free water or 0.1M NaOH. These were placed in a beaker of boiling water that was allowed to cool naturally for 5 minutes. I found that the water method was much more robust (6/6 compared with 2/6) in detecting the Maize 10kd gene when 2µL of

“extracted” sample was used as a template for a 10µL LAMP reaction at 63 °C for 60 minutes. (**Fig 3.2.1 C columns 1-3 compared to columns 4-6**).

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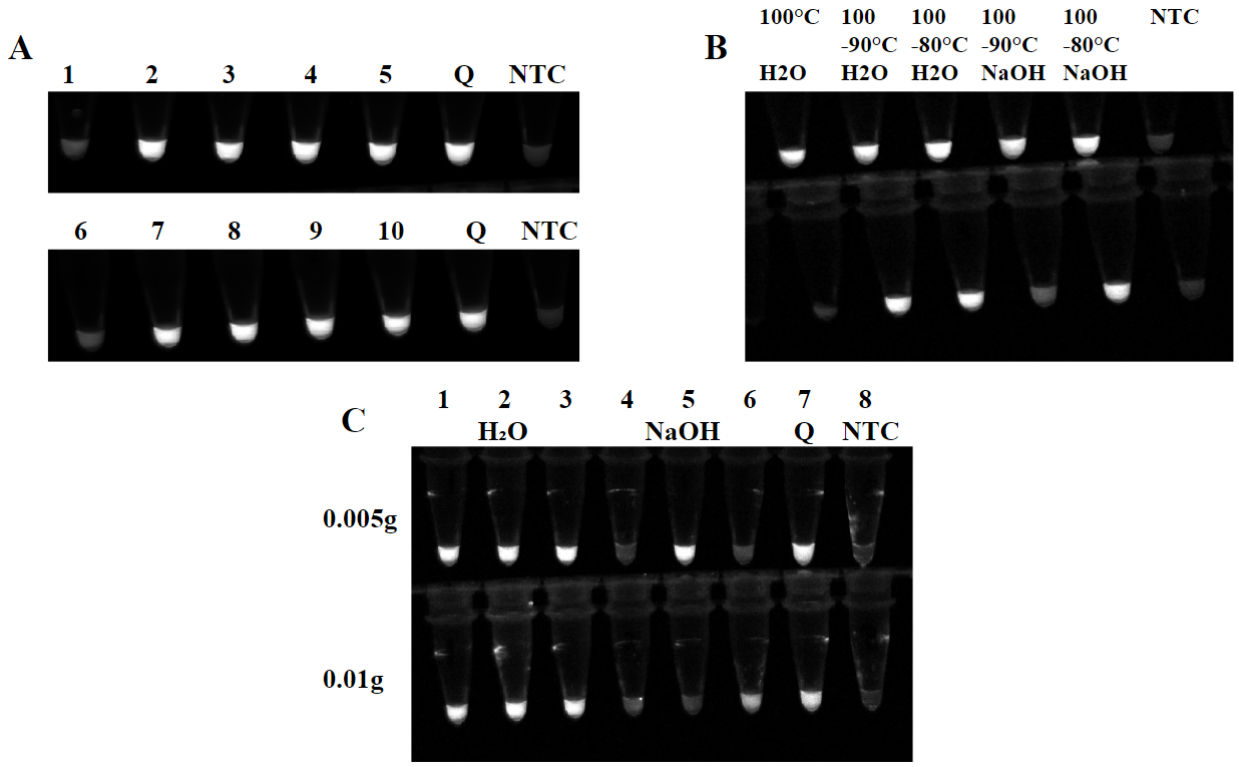


Figure 3.2.1 I developed and tested simple 5 minute DNA extraction protocols. A) LAMP results with 10kD maize specific primers on CRM maize using different simple DNA extraction protocols: 1 and 6 only grinding in water. 2 and 7 grinding then 95 °C with 0.1M NaOH for 17 minutes. 3 and 8 grinding then 100 °C with 0.1M NaOH for 5-10 minutes. 4 and 9 grinding then 100 °C with 0.25M NaOH for 30 seconds, then neutralized and heated again. 5 and 10 grinding and then boiling for 5 minutes with water. Q control using the QIAGEN DNeasy mericon Food Kit. NTC No template Control. **B**) LAMP results of the 10kD primers on maize using the 2 simplest DNA extraction protocols (i.e. Fig 2. A, 3 and 5) with different heating regimes. **C**) LAMP results of the 10kD primers on maize based products purchased from the supermarket. Different amounts of grinded Tortilla chips (columns 1 and 4), 3D Bugles (columns 2 and 5) and Cheese puffs (columns 3 and 6) were added to either molecular grade water or 0.1M NaOH and then placed in a cup of boiling water for 5 minutes.

Further testing was done, to make sure this extraction method was sensitive enough and worked with multiple primer sets and food samples. I tested pre-defined mixes of GMO to non-GMO certified reference material of both soy and corn with our new

method using primers that look for a common GMO marker and a common plant marker. I was able to reliably detect 0.5% GMO content with the 35S QUASR primer set, which is below the 0.9% threshold the European Union places for labeling (**Fig 3.2.2 A**). Our method was also tested on a variety of processed dry foods, with a high corn or corn flour content, with these 2 primer sets. The results were consistent with the labeling on the packages (**Fig 3.2.2 B)**.

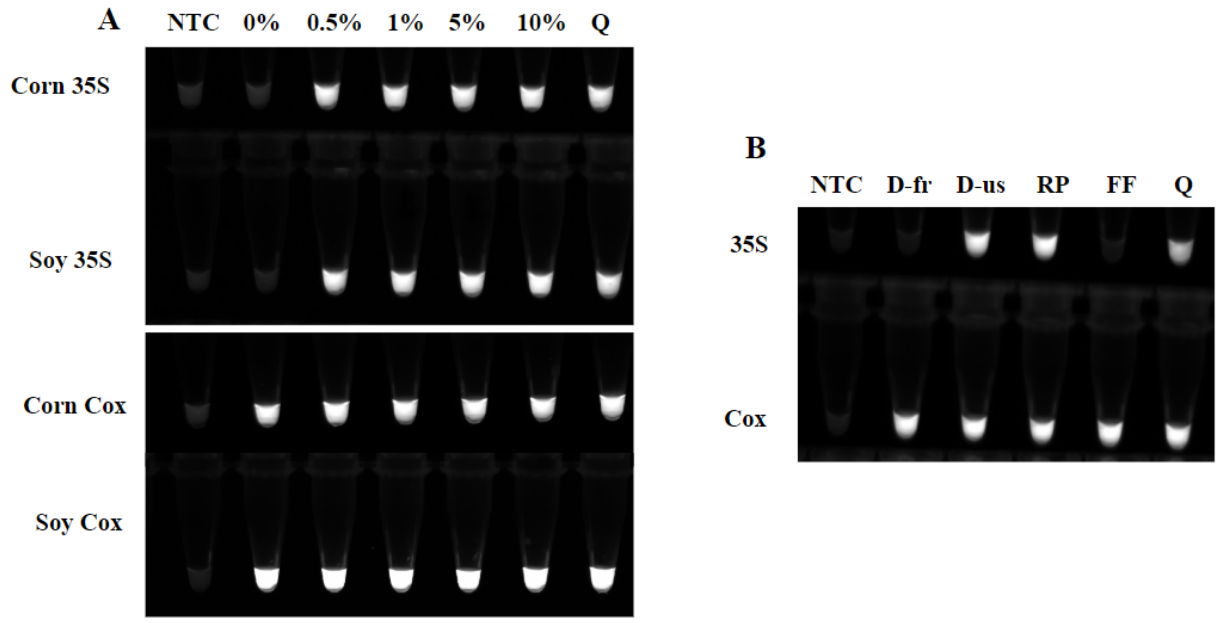


Figure 3.2.2 This novel simple DNA extraction method works with a variety of samples and primer sets: **A**) LAMP results on 0,0.5,1,5,or 10 percent GMO content, on both Maize and Soy (upper and lower rows) using primer sets targeting either the transgenic marker CAMV 35S promoter or the common plant mitochondrial gene COX. DNA extraction was done using our simple method of only grinding then boiling for 5 minutes, except Q where the QIAGEN DNeasy Mericon Food Kit was used. NTC No template Control. **B**) LAMP results using the same primer sets, but on real food samples. D-fr Doritos purchased in France. D-us Doritos purchased in the United states. RP Reese's Pieces morning cereal (contains GMOs). FF Frosties morning cereal.

3.2.2 Lyophilization (Freeze-drying) of the QUASR LAMP reactions

One limitation of the LAMP method (and molecular biology in general) is that the enzyme can denature, and thus, is usually stored in a freezer, and distributed on ice or even dry ice. This complicates and increases the costs associated with transport,

storage, and usage. In order to facilitate adoption and dissemination of this kit/ method to as wide a public as possible, storage and shipment at room temperature is highly advantageous.
To this end, I experimented with freeze-drying the QUASR LAMP reaction mixtures. According to (Pack & Deng, 2008), the problem with lyophilizing the enzyme comes from the glycerol in the buffer it is supplied with. I was able to acquire some glycerol free polymerase which I mixed with dNTPs, the primer mix, and trehalose which helps to stabilize the enzyme (Colaço et al., 1992), and freeze-dried the reactions. After 6 weeks at room temperature (or above) in a dark drawer a mix of isothermal amplification buffer, $MgSO_4$, Betaine and water were added, along with different samples. I found that the reactions gave the correct results (not shown), with the same brightness and speed as the “fresh” reaction.

The isothermal amplification buffer and $MgSO_4$ are usually stored at -20 °C and betaine at 4 °C which defeats some of the purpose of freeze-drying the reaction. I mixed these with water at the appropriate concentrations and left this rehydration buffer for 8 weeks at room temperature. I then tested again the freeze-dried reactions tubes after 8 weeks with the rehydration buffer and found they work just as well as when all components were added from freshly thawed stocks (**Fig 3.2.3)**. We have purchased our own freeze dryer and successfully used it to lyophilize hundreds of reactions in 96 well PCR plates, which have worked even after more than 3 months, and have traveled to multiple countries, without the need for ice (See next chapter).

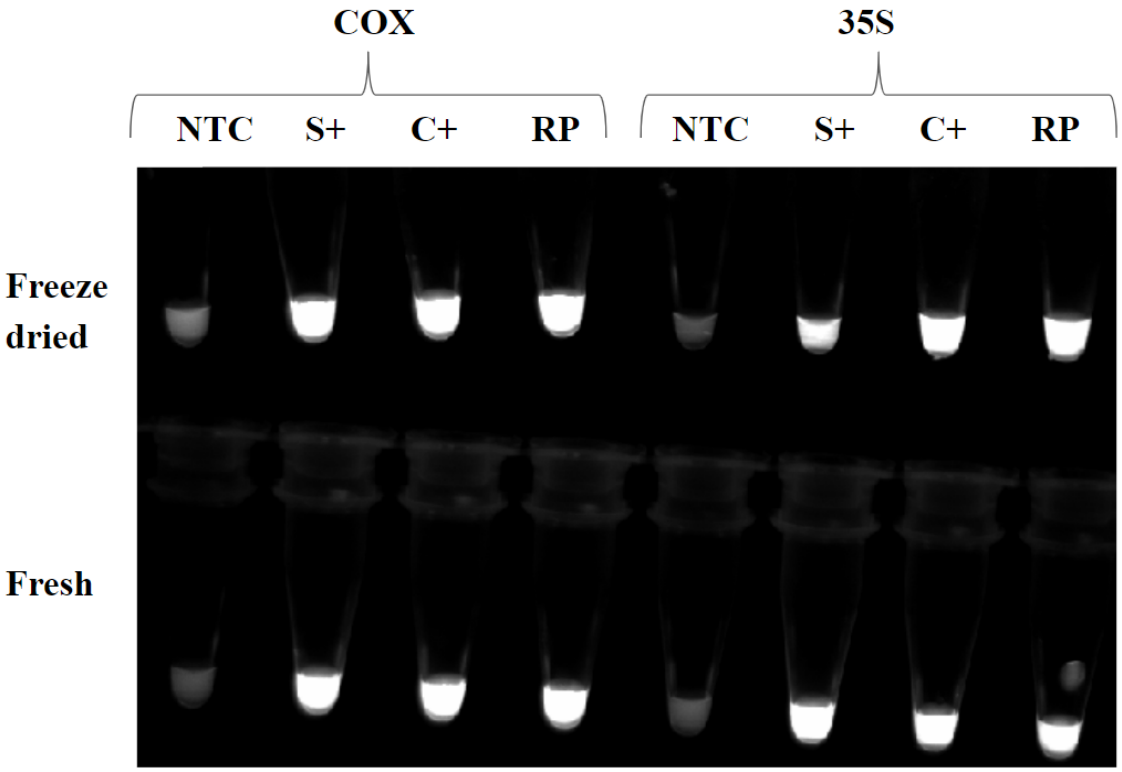


Figure 3.2.3 Lyophilization (Freeze-drying) the enzyme, primers (including the fluorescent/quenching pair), and dNTPs works and allows storage of at least 8 weeks at room temperature. Results of LAMP reactions of freeze-dried mix, to which a rehydration buffer made from isothermal amplification buffer, MgSO4, and water was added, it was also stored at room temperature. NTC no template control S+/C+ GMO soybean/maize, RP Reese's Pieces morning cereal.

3.2.3 Development of an affordable, easy to build and use open hardware fluorescence detector.

All the figures above were made using the ChemiDoc™ MP (Bio-Rad), which is equipped with expensive optical instruments such as the filters and cameras. The paper by (Priye et al., 2017) developed a LAMP box to allow detection using a smartphone anywhere, but this still used expensive optical filters and costs more than 100$. In order to scale this project, we needed to lower this price significantly. As can be seen in **Fig. 3.2.4 A,** the common fluorophore FAM which we decided to use has a maximum of absorption at 495 nm and an emission peak at 520 nm. We tested different blue LEDs such as that in **Fig. 3.2.4 B**, which usually peak at 470 nm, to excite FAM and then looking at the result through plastic gel lighting filters

with appropriate optical properties such as that in **Fig. 3.2.4 C,** (similar to 520 nm longpass filters) available worldwide from Lee filters (used for stage lighting and such: http://www.leefilters.com :"We provide high-quality lighting filters in a vast range of colors that are accurately consistent from batch to batch, long-lasting and dependable. Every filter is checked against a scientifically generated set of parameters."). We also found that adding a blue excitation filter helped to narrow the wavelength band at which the LEDs emitted and produced clearer results **Fig. 3.2.4 D**. These easily allow discrimination of positive/negative results both by eye and camera (See **Fig. 3.2.5 D**), and cost less than 1 cent per 10cm x 1cm strip

To further facilitate the use by laypeople, we designed and built a custom PCB that has the correct spacing of LEDs to shine one LED directly on each tube in a strip of standard PCR tubes (**Fig. 3.2.5 A**). The PCB holds 8 LEDs and a resistor for each one, a fuse to protect the components, along with an optional capacitor to smooth the power that is supplied by a standard 5V microUSB cable. It was also built to be modular so that in the future two could be put together, each with different colored LEDs or filters and the tubes in the middle such that depending on the direction you are looking from you will see the results of different targets/primer sets (see section 2.3.4). The PCB was placed into a custom-designed frame that can be built by laser cutting either opaque plexiglass/wood or cardboard (**Fig 3.2.5 A and C** respectively and Appendix B). All the different parts connect to the "legs" or side panels. The PCB is snapped into the bottom, the different filters slide into the sides, a view-hole connects to the front and a tube holder to the top which places the tubes in the correct position above the LEDs (**Fig 3.2.5 B**). The current design, at small scale costs around 2$ in total, this can go down dramatically at larger scales. The entire "detector" is open-source, and the designs and documentation are posted online to further allow sharing, building and improving by anyone interested (https://gmodetective.com/resources/ and https://github.com/MakerLabCRI/GMODetective-Detector). I have also experimented with different options that will allow people to receive a pre-soldered version using SMD (surface mounted devices) which would lower production and component prices even further.

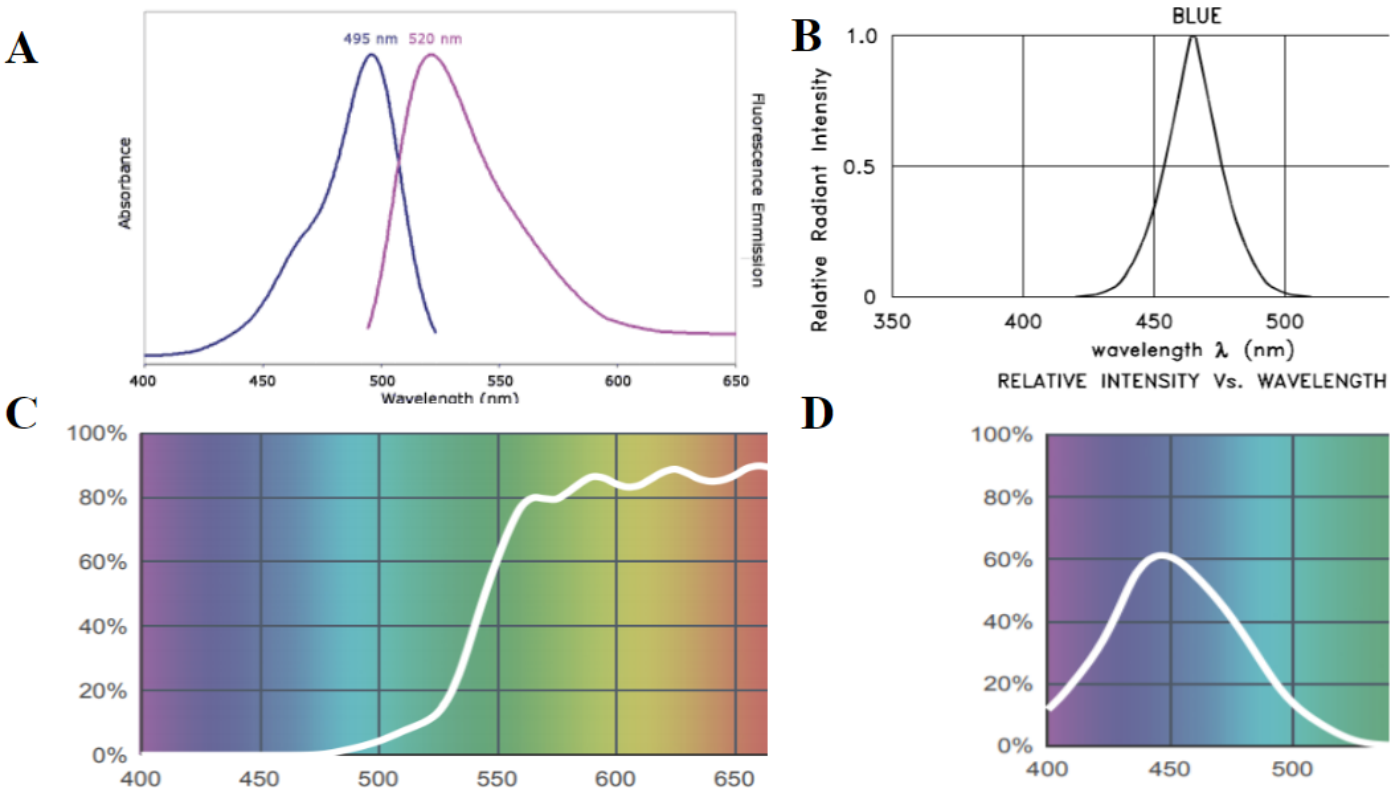


Figure 3.2.4 Detection of FAM fluorescence utilizing ultra-affordable LEDs and plastic gel filters. A) Excitation and emission profile of the FAM fluorophore. B) Emission profile of a typical blue 470nm 5mm round LED. C and D Light transmitted (Y%) for each color wavelength (nm) through orange and blue plastic gel filters. Adapted from: (*Complete LEE Colour Range*, n.d., *Integrated DNA Technologies*, n.d.; LITE-ON Technology Corp. / Optoelectronics, 2013)

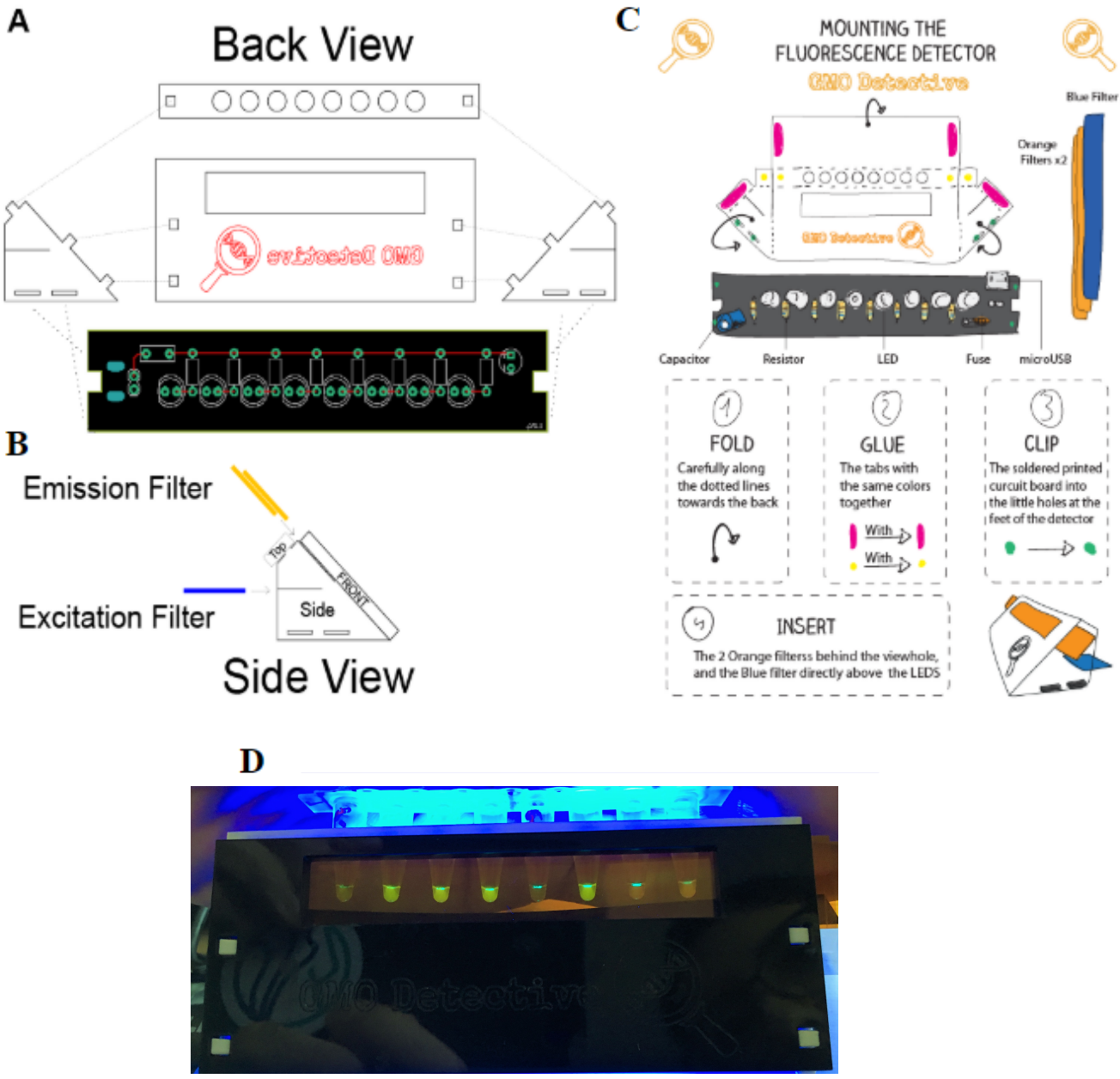


Figure 3.2.5 Easy to build and use, open hardware fluorescence detector. A) Back view of the detector along with the custom-built PCB with correct spacing for LEDs as well as places for resistors, a capacitor, optional fuse, and 5V MicroUSB connector. **B**) Side-view schematic of the detector.**C**) Cardboard version of the detector along with mounting instructions **D**) The detector in use at one of our workshops.

3.2.4 Isothermal heating experiments strategies and hacks.

Another important part of the protocol is heating the reaction to 63 °C for ~an hour. I first designed, tested and built an Arduino based heating system with both resistors and thin film polyimide Kapton® heaters as heat sources. These were sensed by a thermocouple, and controlled by a proportional–integral–derivative controller (PID controller) with Arduino code. This setup was able to amplify DNA, but we quickly realized that in order to do workshops with classrooms and larger groups this solution does not scale well to large numbers of reactions. It also suffers from problems of uniformity and evaporation. Depending on where the workshop is done some might have access to water baths, heating blocks or PCR machines which are all ideal but cost hundreds to thousands of dollars. We tested other more affordable alternatives; Commercial portable sous-vide precision cookers function just as well but at a fraction of the cost (50-200$) (**Fig. 9-4**) and are able to maintain a constant 63°C (∓0.5 °C, data not shown) for hours. Sous vide is a relatively new way of cooking where food is placed in a vacuum-sealed bag and then kept at a constant temperature, thus never overcooking or drying out, even if left for a very long time. We successfully used these as a heat source for LAMP reactions in a variety of locations. Multiple open hardware projects exist online if people would like to build their own water bath/sous vide machines (https://www.instructables.com/howto/sous+vide/). We also tested commercial wax heaters, which can be slightly cheaper, but unfortunately, these models do not actually keep a constant temperature but vary by ∓2-6 °C during operation.

3.2.5 Packaging into a modular workshop for laypeople and students.

We designed the simplest experiment that could allow correct identification of samples which may contain a modified crop (**Fig 3.2.6**). The first four tubes contain 8µL of the mastermix (enzyme buffer nucleotides and primers premixed) for detecting a common plant gene, this is in order to control for successful DNA extraction. The second 4 tubes contain the mastermix for the GMO marker. The first

and fifth tubes are negative controls, where we place 2μL of water in each. The 2nd and 6th tubes are positive controls, 2μL of either PCR product or DNA extracted with a commercial kit (or water if using the freeze-dried version with controls already included). The 3rd and 7th tubes are the first sample and the 4th and 8th are for the second sample.

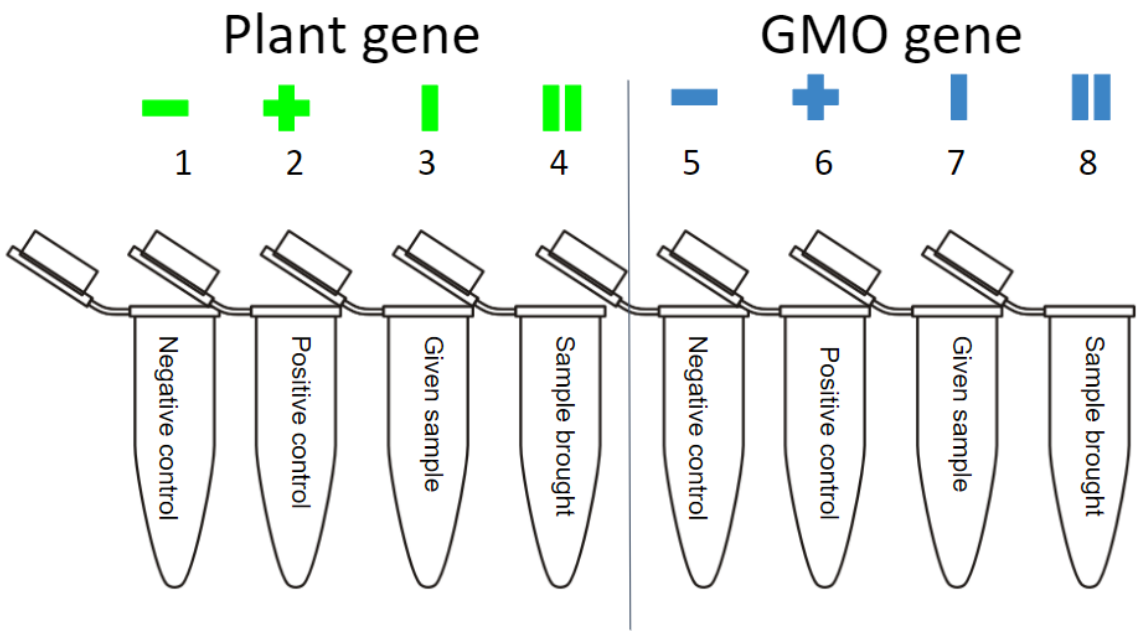


Figure 3.2.6 Design of the simplest LAMP experiment to detect a common GMO marker in 2 different samples.

I put all the previously discussed pieces together to create a workshop that can be done in as little as 2 hours, and did preliminary tests with diverse groups. The workshop usually begins with a brief introduction to DNA, base pairs, the central dogma, and a short TED-ED video (https://www.youtube.com/watch?v=z9HIYjRRaDE) on where genes come from, and how they change and evolve over time. This leads us to a short description of PCR, its incredible power but also its shortcomings, regarding cost, time and analysis of the results. Next is an overview of the protocol (**Fig. 3.2.7**) and an explanation of both how to use a micropipette and what exactly goes into each tube and why. Then the hands-on part begins, (**Fig. 3.2.7**) participants crush samples using a pestle, add water and place samples in a beaker/cup of boiling water for 5 minutes. The right mixes are added to PCR tubes and then the samples/controls too. These are then placed at 63°C for an hour to incubate.

Depending on the setting and audience this hour can be used to discuss different issues such as: How the things we eat today have greatly diverged from their ancestors. Different ways of genetically modifyng crops; traditional breeding vs (Mutagenesis vs RNA interference vs Transgenics). Transgenics in other contexts; insulin, artemisinin, chymosin or immuno and gene therapy for example. Types, uses, and growth in the use of transgenic crops today and in the future. When and how should we use transgenics? Etc. It can also be used to take a break and/or eat a meal. The results are stable for weeks, even at room temperature, so the analysis can also be done on a different day.

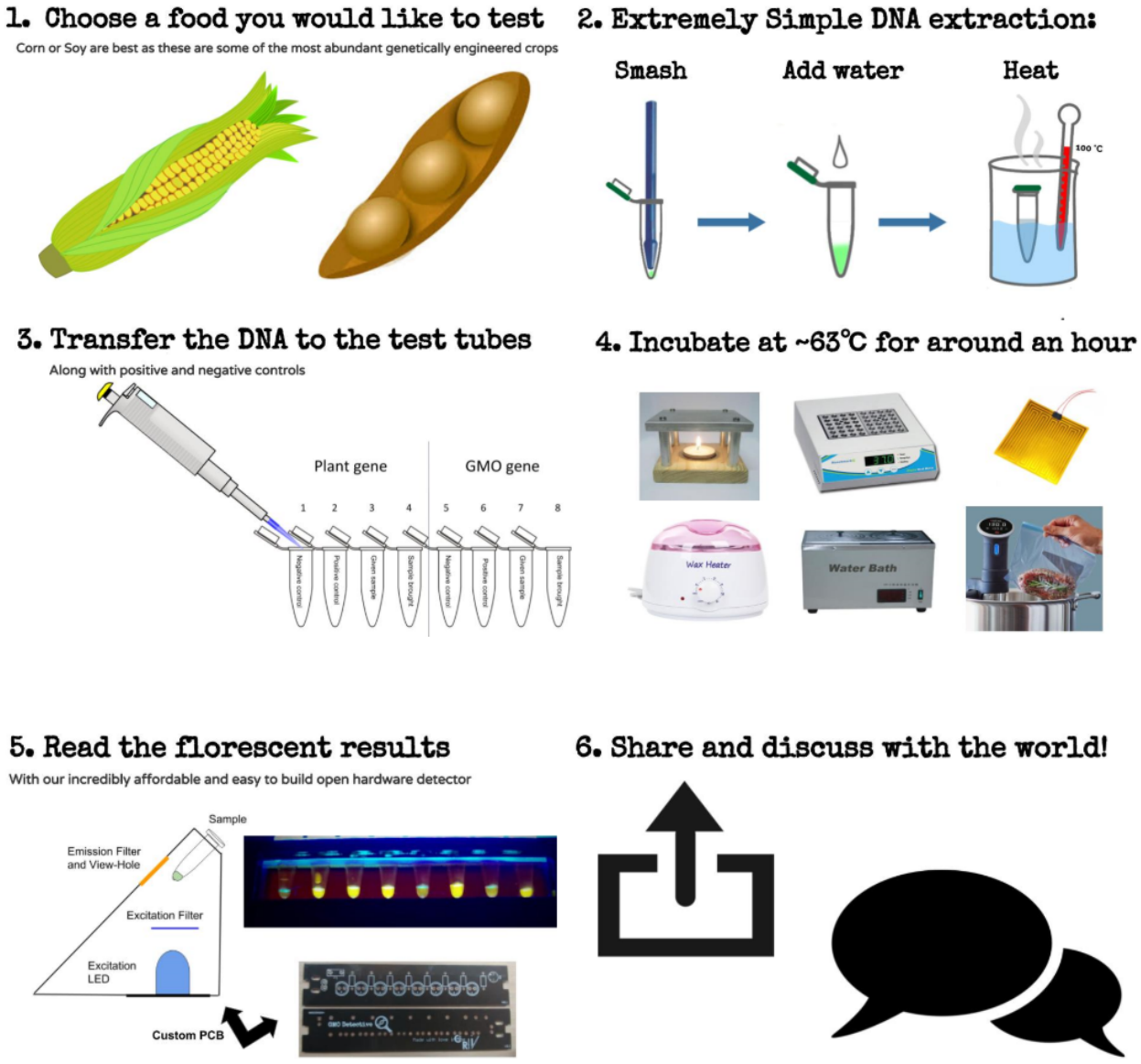


Figure 3.2.7 Workshop experiment overview.

3.2.6 Lowering costs by an order of magnitude.

A major hurdle to accessibility to molecular biology remains budgetary. Especially in lower resource settings such as developing countries, some schools, and marginalized communities. Through a variety of strategies, I was able to lower the cost of a LAMP experiment, especially for a big group setting such as in a workshop or class, by an order of magnitude, from what would typically be spent in a lab. The standard NEB warmstart LAMP mastermix costs €2 per reaction, and this is the most affordable LAMP mastermix on the market. By sourcing the different components at a medium scale (enough for hundreds to thousands of reactions) from the most affordable suppliers, and optimization of the reaction conditions (see section **2.3.5**) I was able to lower the cost of an experiment (8 reactions see **Fig 3.2.6** and next section for experimental design) from almost €17 to less than €1.5 (**Table 3.2.1**). Consumables were also bought directly from chinese manufacturers at scale, and different brands tested. We found versions of all necessary products, which work just as well but again at a fraction of the cost from scientific suppliers such as Sigma Aldrich or Amazon. These all have free worldwide shipping, but this can take weeks, depending on location. All consumables (mainly plasticware and gloves to protect from contaminating the experiment) needed can be acquired for ~€0.5 per experiment. (**Table 3.2.2**).

Type	Name	Standard LAMP	GMO Detective	Optimized
WetWare	Enzyme	€16.00	€0.97	€0.61
	MgSO4		€0.02	€0.02
	dNTPs		€0.36	€0.41
	Buffer		€0.03	€0.03
	Primers	€0.65	€0.26	€0.19
Total		€16.65	€1.65	€1.27

Table 3.2.1 Cost of WetWare for a LAMP GMO detection experiment (8 tubes/reaction)

Type	Name	For 1 Workshop (16 ppl)	# Needed for 1 experiment	Aliexpress		For 1 Workshop (16 ppl)	For 1 Experinment
				# of pieces/lot	Price		
Consumable	1,5 mL microube	40	4	100	€2.10	€0.84	€0.08
	PCR 8 tube strip	10	1	200	€17.06	€0.85	€0.09
	Pestles	20	2	1000	€64.55	€1.29	€0.13
	Pipet Tips	100	10	1000	€9.07	€0.91	€0.09
	Pipet Tip in Box	4	1	1	€3.98		
	Gloves	20	1	50	€8.00	€3.20	€0.16
	Aluminium foil	1	0.1	30	€1.75	€0.06	€0.01
					Total	€7.15	€0.55

Table 3.2.2 Cost of Consumables for a LAMP GMO detection experiment (8 tubes/reactions) and a workshop (8 experiments)

In regards to reusable tools, which includes the fluorescence detector, isothermal heater and micropipette significant reductions were also achieved. As discussed the most affordable fluorescence detector on the market, the P51™ Molecular Fluorescence Viewer from MiniPCR costs 25$, our similar open source version can be built for less than €2 (Table 3.2.3). And finally a precise Sous vide heater can be acquired for €50 (Biolomix Sous Vide Cooker), while the cheapest, and yet precise enough 2-20uL micropipettes I have found cost €20 each (JOANLAB 2-20ul Single Channel Adjustable MicroPipette https://www.aliexpress.com/) For more details see Appendix C and D.

Type	Name	For 1 Workshop (16 ppl)	# for 1 detector	Price/unit	For 1 detector	For 8 detectors
Electronic component	PCB(Printed Circuit Board)	8	1	€0.20	€0.20	€1.60
	LEDs 468 nm	70	8	€0.15	€1.20	€9.60
	Resistor 68 ohms	70	8	€0.01	€0.08	€0.64
	Capacitor 10 µF	10	1	€0.05	€0.05	€0.40
	Fuse 0,5 A	10	1	€0.20	€0.20	€1.60
Consumable	Solder	1	0.05	€2.93	€0.15	€1.17
Plastics	3mm plexiglass	400cm2	120x82mm	€7.53	€0.03	€0.22
	Orange filters	400x110mm	40x110mm	€6.14	€0.05	€0.36
	Blue filters	150x110mm	15x110mm	€6.14	€0.02	€0.14
				Total	€1.97	€15.73

Table 3.2.3 Prices of the GMO detective Detector Parts.

3.2.7 Preliminary tests and iterations of the workshop in the lab.

An important aspect of this project is the use of DNA detection as an educational tool. We did preliminary tests of the workshop on diverse groups in our lab in order to verify and improve the workshop. The first was a longer extended version with a

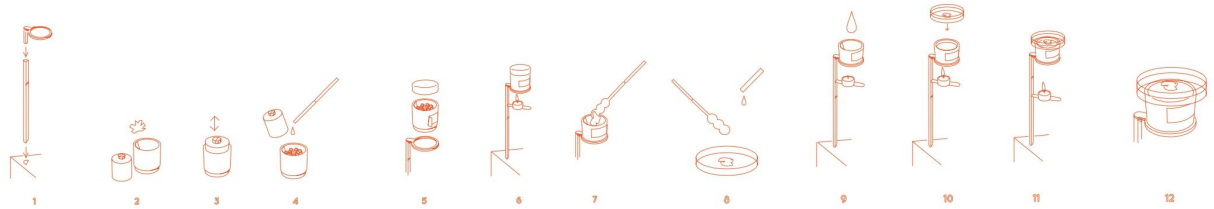
small group of 4 motivated 16 year old students, looking for an extracurricular science project over the course of three 3 hour meetings. In the first meeting, they were introduced to different concepts and given reading to do about LAMP, PCR, GMOs, and fluorescence, and told to come back with questions, then they watched me perform the experiment. In the second and third meetings, they conducted the experiments themselves on food samples they brought in. After this initial success, we wanted to expand to shorter timeframes and larger groups. We conducted a 3 hour workshop with a group of 16 master students in design, who knew very little about biology and had no experience in the lab, but were interested as they were taking part in a Biodesign course we gave (see next section). The next was with a small (6) internal group of researchers from the CRI, some biologists and others not at all, who participated in a short 2 hour version. And lastly a group of high school students who came for a longer version in which they built the detectors in the morning in our makerspace and then did the biology part in the lab after lunch. These iterations, along with the feedback we gathered from them allowed us to improve the preliminary design of the workshop. We learned that most participants found pipetting to be the most challenging part of successfully doing the experiment. We also learned where to put emphasis and what can be more difficult for different audiences to understand. Mainly how DNA amplification works, as well as, what exactly is in each tube and why, more emphasis was then placed on these issues. We also improved the timing and pace, 2 hours was found to be rapid but possible for just the biological part, 3 hours allows more time for breaks and discussion. Lastly, we improved our protocol and made it much more visual and color-coded with every step written out and also illustrated, which also helped participants understand better what they were doing (See Appendix E)

3.2.8 Bio Design Challenge course and Minima

Students are not the only group that we want to work with, and these topics are of intense interest to a wide audience. The workshop was also done with a group of designers in a Biodesign class for designers and biologists together, in the context of the Global Biodesign Challenge where the topic was chosen to be DNA. Out of the five projects the interdisciplinary group I mentored, went on to win the local competition in Paris (see (Chardronnet, n.d.) in french) by reimagining food DNA

detection for a kitchen or home. Instead of using the usual scientific single use plastic tubes and electronic devices, Minima-the elementary biology lab (See (Lherry, n.d.) and https://vimeo.com/276128398) built a colorimetric LAMP kit using a familiar set of ceramic and glass tools for sensing DNA in food. With isothermal heating accomplished by using a candle (**Fig 3.2.8)**. Minima became the first French team to compete and also reach the final (8 out of more than 100 teams) at the Museum Of Modern Art (MoMa) in New York City with their project inspired by this PhD.





Minima is a set of ceramic and glass tools for sensing DNA in food. Everything we eat contains a specific DNA signature. Biologists have found a way to detect those signatures with a blend of enzymes, nutrients, and specific DNA sequences, producing a color-change reaction. We have packaged this cutting-edge biotechnology into a simple piece of kitchen equipment. The result is an next-generation household appliance : simple, affordable, familiar. Minima will empower anyone who who keeps a specific diet and everyone who is curious about food.

A project by Esther Bapsalle, Pauline Jourdan & Naiane Ribeiro Rios
minima.biodesign@gmail.com / minima-biolab.org

Figure 3.2.8 Minima-the elementary food biology lab kit and instructions as designed and built by an interdisciplinary team of designers and biologists mentored by me and who went on to be the first French team to reach the finals at the international biodesign challenge at the Museum Of Modern Art (MoMa) in NYC.

3.2.9 Website and documentation for the public

In order to increase visibility and allow people to learn about the project, as well as recreate it themselves I taught myself how to and then built a dedicated website: https://gmodetective.com/ . It is updated as the project progresses and currently has multiple pages including a general overview and explanation of the project, detailed scientific background along with links and images. A variety of open shared resources such as protocols, presentations in both french and english (soon to be spanish too) and detailed hardware/wetware guides and files (See Appendix C and E). In addition to a friends page with links to other projects, places, organizations and other relevant resources. I am building a forum and also include a way to upload your results in addition to ways to contact us (**Fig 3.2.9)**.

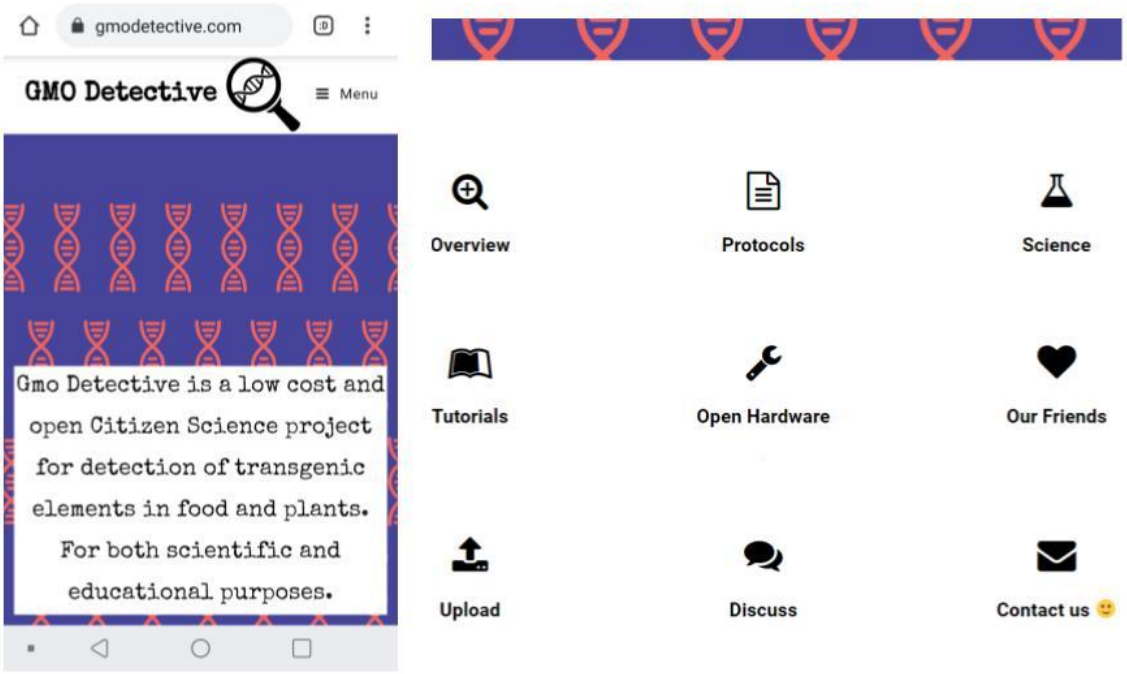


Figure 3.2.9 GMODetective dedicated website on both mobile and desktop platforms

3.3 Discussion

The promise of LAMP assays has always been that they are simpler, faster and require less training and instrumentation (Yasuyoshi Mori et al., 2013; Y.-P. Wong et al., 2018). Although this has been achieved in numerous cases before, most solutions found until now do not scale in a way such that tens to hundreds of tests can be performed at the same time, especially by laypeople. I improved on existing LAMP assays by making them more robust, false-positive resistant, detectable using fluorescence and stable for at least two months at room temperature using lyophilization. This assay was coupled to a simple 5 minute DNA preparation protocol which only requires one 1.5ml tube, a pestle and water. I also developed open-source hardware and 'hacks'. Our extremely affordable, easy to build and repair detector allows for a straightforward and instantaneous readout of the results by eye or camera. This piece of open hardware could also be used for other fluorescent assays, especially in low resource settings. Importantly I was also able to lower the price of both the reactions and necessary hardware by at least an order of magnitude from what these usually cost in the lab, so that it could be done anywhere by anyone. (prices per experiment: ~0.5€ for consumables ~1.5€ for 8 tubes of reagents while for reusables ~2€ for the detector ~20€ for the micropipette and ~50€ for the heater).

This was all packaged into an open modular workshop that can easily be adapted to different audiences. Building the detectors from scratch even with people who have never soldered before takes less than an hour. The biology experiment takes less than one hour even with people who have never used a micropipette. The hour of incubation can be used to discuss deeper issues, or results can be analyzed at a different date as they are stable for weeks. Everything you need to both build the hardware and do the biological experiment for 20 participants easily fits in a carryon size trolley bag (for a complete list of materials see appendix D). All the different aspects of this work are open source and detailed guides are available online such that anyone can recreate this themselves, some have already done so engaging with the public and students (see next chapter). I purposely tried to make the experiment and all other aspects as easy and open as possible in order to allow the greatest

number of people to be able to participate, learn or expand upon for their own uses. This work along with initial successes we had in the lab with diverse groups gave us the confidence to take the developed workshop out into the real world (See next chapter).

Soon after the work on DNA extraction was conducted and after the preliminary workshops had begun, an interesting paper was published titled: Nucleic acid purification from plants, animals, and microbes in under 30 seconds (Zou et al., 2017). Zou et al. describe 2 simple DNA extraction protocols which they patented in Australia that might be useful in contexts such as ours. Briefly, they propose using cellulose based Whatman No.1 paper discs or dipsticks in order to extract DNA from samples. The first protocol first grinds the samples using a pestle in a lysis buffer, then a 3mm paper disc is placed inside for one minute, this disc is then transferred to a wash buffer for another minute followed by transferring the disc directly into a PCR or LAMP reaction. The other “30 second” protocol is similar but more rapid and pipette free. The sample is added to a tube containing a lysis buffer and 2 ball bearings which are used to lyse the sample, then a Whatman paper dipstick is dipped 3 times to bind DNA. the dipstick is then plunged 3 additional times into a wash buffer and then dipped 3 more times into the amplification reaction. They also show similar results using commercial paper towels. I have not yet tried these methods as our protocol was almost as fast but simpler and required only water and 1 tube, instead of 2 tubes and different buffers. It seems the disc method has recently been adapted for use in high school settings, with DNA barcoding efforts (dnabarcoding101.org, n.d.), where they report a success rate of 30-85%, with terrestrial plants corresponding to the higher range. This method might be useful with foods/plants in which our extraction does not work well, or in the future if looking for other targets, but has not yet been tested or compared to the methods developed here.

Another aspect of making detection of DNA more accessible is the use of micropipettes. These are not especially affordable (20$ is the best I could find for 2-20ul pipettes suitable for our purposes) or easy to use. On the one hand, this is usually the most difficult part for lay people when doing the experiment , but on the

other hand this is also commonly where people feel they have learned a new skill, or makes them feel like scientists (See next chapter). As currently set up, the GMO detective experiment requires transferring 3 different volumes :
60ul for the DNA extraction.
8ul of buffer or mastermix to reaction tubes
2ul of each sample and or control
A 2-20ul micropipette can do all of these and this is also why these volumes were chosen. We did experiment with disposable glass microcapillary pipettes, but these were extremely hard to use even for trained personnel. Especially for both 2ul and 8ul volumes and when the extracted DNA samples can be of different viscosities.
A promising avenue for future research could be to lower the volume of the reaction down to 6ul total and then use pre-pipetted 5uls of buffer or a fixed volume disposable pasteur pipette and 1ul bacterial loop for the sample. Other solutions could include custom 3d printed loops for different volumes or preallocated micro tubes, tiny bags that need to be cut and emptied, or some form of droplet (e.g. wax encapsulation) instead of pipetting.

Chapter 4:

GMO detective as a tool to demystify, empower, educate and inspire

4.1 Introduction

Rapid advances in molecular biology as well as in the reading, writing and manipulation of DNA and the corresponding implications of genomics research are finding their way to everyday life. Major breakthroughs range from medicine to forensics, biofuels, vaccines, mitigation of pollution and genetically modified agricultural products, bacteria and even humans (Arenas et al., 2017; Gao et al., 2019; Kirksey, 2020; Lim, 2009; Shanmugam et al., 2020; L. Tan & Sun, 2018; Wurtzel et al., 2019). Like any techno-scientific advance these each bring their own choices, dilemmas and controversies (Al-Rodhan, 2015). In order to empower current and future citizens to deal with these personal and societal decisions in a constructive way, deeper understanding and more engagement would be beneficial (McPhetres et al., 2019; Potter et al., 2017; Verhoeff et al., 2009). With this project we aim to improve scientific literacy (Laugksch, 2000) in a constructivist (Ackermann, 2001) and active (Freeman et al., 2014; Prince, 2004) interdisciplinary (Van Hecke, 2002), hands-on learning by doing (Satterthwait, 2010), making (Martin, 2015), seeing (Stark et al., 2018) and discussing (Goldschmidt et al., 2016) way.

4.1.1 Hands-on experiences in schools

For more than a century science education has argued for the importance of practical experiences in the learning and practicing of science (Rudolph, 2014). The first large report on the state of laboratory experiences in the United States ((NRC 2006), America's Lab Report: Investigations in High School Science. Committee on High School Science Laboratories) identified a number of science learning goals that have been attributed to laboratory experiences, including:

- *Enhancing mastery of subject matter.*
- *Developing scientific reasoning.*
- *Understanding the complexity and ambiguity of empirical work.*
- *Developing practical skills.*
- *Understanding of the nature of science.*
- *Cultivating interest in science and interest in learning science.*
- *Developing teamwork abilities.*

All of which are important topics in the learning of science, especially in school. Some of the identified factors which make hands-on activities so effective, include peer interaction through cooperative learning, object-mediated learning and embodied experience (Satterthwait, 2010). Studies do show that mixed-modality presentations (for instance, combining auditory, kinesthetic, and visual techniques) improve results in a variety of subjects (Presti, 2015) and is more likely to enhance learning in a heterogeneous student population (Bransford et al., 2000).
Arguments against teaching science as a passive collection of facts go back more than a century where Dewey lamented those teaching science presenting the subject as ‘‘an accumulation of ready-made material with which students are to be made familiar’’ and ‘‘not enough as a method of thinking’’ (Dewey, 1910). These calls have continued throughout the years, such as a similar and more pointed critique of (Peter Volpe, 1984):

“Public understanding of science is appalling. The major contributor to society's stunning ignorance of science has been our educational system. The inability of students to appreciate the scope, meaning, and limitations of science reflects our conventional lecture-oriented curriculum with its emphasis on passive learning. The student's traditional role is that of a passive note-taker and regurgitator of factual information. What is urgently needed is an educational program in which students become interested in actively knowing, rather than passively believing.”

Which is still as relevant today (Michael, 2006) as it was more than 35 years ago before I was born.

These critiques and calls have spurred decades of research into more active forms of learning (generally defined as any instructional method that engages students in the learning process (Bonwell & Eison, 1991)), as a preferred pedagogy (see Freeman et al.(2014) and Prince (2004) for a review and meta-analysis respectively). Along with the related methods of inquiry based learning (reviewed in Pedaste et al., 2015; Rönnebeck et al., 2016)) and problem based learning (Albanese & Mitchell, 1993; Ward & Lee, 2002) paradigms which are more interdisciplinary and focus on guided self inquiry. Other active approaches have emerged such as the constructionist approach which emphasizes 'learning to learn', and the significance of making things in learning (Ackermann, 2001; Harel & Papert, 1991). Which has recently given rise to paradigms such as design-make-play learning methodologies (Honey & Kanter, 2013), that become even more relevant with the rise of the maker movement (Blikstein & Krannich, 2013; Halverson & Sheridan, 2014; Martin, 2015)) and help foster student autonomy, which can then self-empower (Quinn & Bell, 2013). In conclusion and closer to our specific subject, (Dirks, 2011) in her review on biology education research states that "Given the overwhelming evidence that active learning improves learning in science, it should no longer be referred to as a pedagogical practice, but rather it should be considered the central dogma of science education".

4.1.2 Molecular biology in secondary schools

(Tibell & Rundgren, 2010) argue that molecular life science is one of the fastest-growing fields of scientific and technical innovation, and that biotechnology has profound effects on many aspects of daily life—often with deep, ethical dimensions. But that the field also poses diverse challenges in learning and teaching especially in regards to content, reasoning difficulties, and communication issues (language and visualization). They suggest that the inability to directly experience the molecular world may explain many of the documented learning difficulties in molecular sciences. Specifically in regards to GMOs even advanced undergraduate biology majors often exhibit wrong notions and explanations, including essentialist reasoning (Potter et al., 2017). Efforts to incorporate hands-on molecular or synthetic

biology in schools (or outside of universities) have been limited by (i) the number of robust systems that can be used as teaching materials; (ii) the need for expensive, specialized equipment and training; and (iii) safety considerations that limit the ability to work with cells or chemicals outside of a laboratory setting (Kuldell, 2007; Stark et al., 2018). Despite these hurdles, a few such examples exist and will be discussed.

The first example I could find, which is now more common, is of the outreach variety (Bouakaze et al., 2010). Where university students bring all the required reagents and modern/expensive equipment into the classroom (or classes take field trips to the university) and conduct the workshop. The experience was a 2-hour PCR-based practical developed around a fictitious criminal investigation with the goal of helping high school teenagers understand theoretical and abstract concepts in genetics and to attract them to biology. Fake plasmid DNA was used as a template, and students received samples labeled victim and either suspect 1 or 2. Students then mixed the DNA and mastermix, ran a fast PCR protocol (30 minutes) during which the principles of this amplification technique and of gel electrophoresis were explained (15 minutes). Subsequently,
students practiced loading samples into agarose gel slots (15 minutes). This time period was also dedicated to discussions with students about their plans after graduation. The PCR amplification products were then loaded into the gels (15 minutes), and while the electrophoresis was running (20 minutes), various applications of PCR were reviewed and illustrated with concrete examples. At the end of the practical, students were invited to discuss the results of the electrophoresis visualized on a transilluminator (10 minutes).
(Bouakaze et al., 2010) found that both students and teachers were quite satisfied with different aspects of the workshops. These experiences seem to have been successful and have continued, but switching to a new design centered around proteins and their structure, not DNA after three years (*OpenLAB, à La Découverte Du Monde de La Recherche - OpenLAB - Université de Strasbourg*, n.d.) reaching thousands of students over the years.

A similar mobile outreach program but at a larger scale has been implemented in the Netherlands called DNA-labs on the Road (dnalabs.nl). Six different Universities/ Research institutions each developed a lab relating to their work. These are

structured so that teachers/schools order a workshop (for ~300€) teachers are expected to give a one hour introductory lesson and a 1-2 hour concluding lesson after, and university students come in between to give the 2-3 hour practical activity. These include a variety of foci from purely bioinformatic, to protein (mis)folding, DNA fingerprinting and producing bioethanol from yeast. The goals of these were stated as 1) Enhancing up-to-date genomics knowledge. 2) Improving the image and attitude towards genomics topics 3) Increasing the notion of societal implications of genomics research (place genomics in a societal context). 4) Invoking enthusiasm and interest in genomics research. Details and an evaluation in English could be found only for one of these labs: 'reading the language of the tumor' (Van Mil et al., 2010). The activity was designed around the context of a diagnostic DNA test on tumor tissue to determine the best treatment for a fictitious cancer patient. Due to technical and legal limitations, students isolate DNA from calf thymus to illustrate the principles of DNA extraction and then use plasmid based fragments to obtain PCR products that simulate the fictitious mutations in three different genes which affect cancer in different ways (loss of apoptosis, overactivation of growth and changes in cell adherence). After this workshop students consider themselves capable of explaining the importance of DNA research in hospitals in the context of cancer diagnosis, and stimulated in them a positive attitude towards DNA research (Klop et al., 2010). They conclude that the DNA lab is a good example of relevant and up-to-date genomics education.

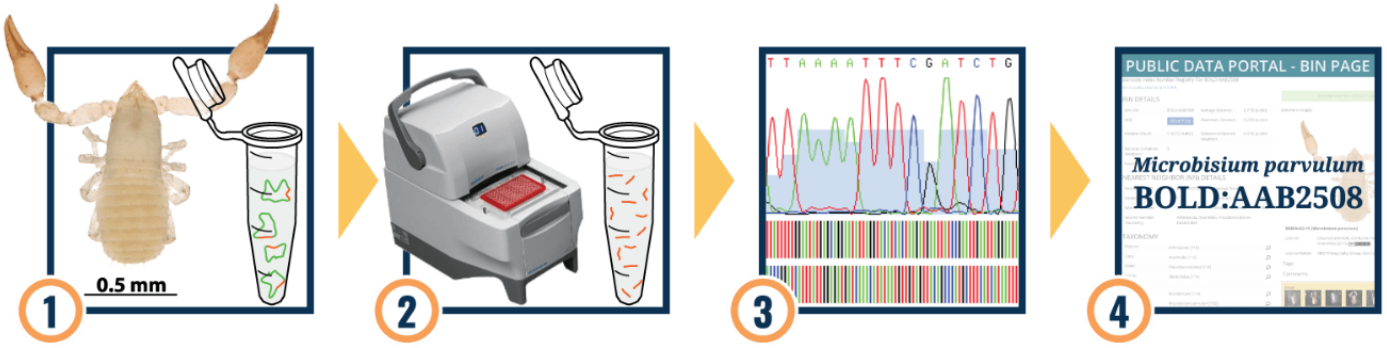
In the United states similar modules have been developed, but without the outreach of students teaching the practicals, and with little to no research on outcomes I could find. The Cold Springs DNA Learning Center invites students for daylong field trips (6 hours) to their labs (32$ per student) and offers mostly PCR based practicals such as PTC: Using a SNP to Predict Bitter Tasting Ability, Forensic DNA Profiling, Extended Human DNA Fingerprinting: Using an Alu Insertion Polymorphism to Study Human Populations and GMO: Detecting Genetically Modified Foods (*CSHL DNA Learning Center*, n.d.). The various materials for these labs are also available through Carolina Biological Supply Company (https://www.carolina.com/) internationally for ~200-300$ per activity. Similar kits are also available from Bio-Rad Laboratories, Inc. (www.bio-rad.com/en-fr/category/pcr-amplification-kits) and miniPCR (miniPCR Team, 2018)/ miniONE (theminione.com) (which are cheaper

and only cost~100$ per kit per class) all of which also sell thermocyclers and electrophoresis apparatus starting at ~1000$.

Recently, a new methodology was described which uses freeze-dried, cell-free extract and a just add water approach to teaching molecular biology named BioBits™ (A. Huang et al., 2018; Stark et al., 2018). These are now being commercialized also by minPCR (https://www.minipcr.com/products/biobits/). Briefly cellular components are harvested from bacterial lysate including polymerases, ribosomes, and transcription factors, to carry out the processes of transcription and translation in vitro, which circumvents the need for specialized, sterile equipment and media to culture living cells. These are supplemented with additional essential components such as nucleotides, amino acids, and energy equivalents, and freeze dried for stability at room temperature. DNA plasmids coding for different proteins can be added. (Stark et al., 2018) express for example a variety of fluorescent proteins. The amount of template DNA or the ratio of 2 different templates can be varied to affect the color. Or modulators such as antibiotics which work on ribosomes can be added to affect translation (A. Huang, 2019). Other DNA inputs include fragrance or hydrogel-generating enzymes. These reactions usually require incubation at room temperature or 37C for anywhere between 8-72 hours. One module is currently available for purchase, BioBits: Central Dogma which includes DNA encoding for a red fluorescent protein, along with an aptamer on the RNA which causes it to glow green when transcribed. This can be observed after ~20 minutes, and the red fluorescence can be observed in the following day/s. This allows visualization of the central dogma of biology easily in a hands-on way in a classroom and costs ~100$ for 32 reactions not including the fluorescence viewer.

Finally in an effort to promote a more authentic student scientific contribution, DNA barcoding seems to be growing in popularity, and to have begun bottom up through a student led project (Schwartz, 2008) identifying mislabeled sushi in new york city.
In DNA barcoding, a short, standardized region of the genome is used to differentiate species. An international effort to ‘barcode’, or create a reference library of barcodes from known species, is ongoing, with 500 000 species already in the Barcode of Life Database (Ratnasingham & Hebert, n.d.), with the aim of reaching 2.5 million species by 2026 (*The International Barcode of Life Consortium*, n.d.). Steps in the

workflow include collection and identification of specimens, DNA extraction, PCR, DNA sequencing and contribution or comparison to a database (**Fig 4.1.1**)



Step 1: Isolate DNA from the sample
Step 2: Amplify the target DNA barcode region using PCR
Step 3: Sequence the PCR products
Step 4: Compare the resulting sequences against reference databases to find the matching species

Figure 4.1.1 The steps of DNA barcoding. From (https://ibol.org/about/dna-barcoding/)

DNA barcoding efforts have been integrated into school systems in different countries and at different age levels, due to the modularity of these steps (Henter et al., 2016). For example younger students focus on collecting specimens but send those specimens elsewhere to generate DNA data such as in the The School Malaise Trap Program in Canada (Naaum et al., 2013; Steinke et al., 2017). Older students participate in more of the steps including the DNA extraction and PCR in projects that for example investigate the diet of an invasive fish (Eble & Pecore, 2019), survey biodiversity in urban, suburban or protected areas (dnabarcoding101.org, n.d.; Marizzi et al., 2018), or barcode large collections from natural history museums (Chiovitti et al., 2019). These programs generally run from two days to two weeks, and a variety of tools have been developed to assist students and teachers in using these methodologies, including an app (http://www.dnabarcodingassistant.org/) and an educational portal (http://www.educationandbarcoding.org/). Both students and teachers generally report high satisfaction from these experiences (Chiovitti et al., 2019; Santschi et al., 2013).

Focus here was on shorter and simpler nucleic acid based modules, more complex week or month long experiences also exist which usually focus on microorganisms.

The Phage Hunters program has been implemented mostly in undergraduate institutes but also with high school students (Hanauer et al., 2006; Jordan et al., 2014). Depending on time and resources participants may isolate a novel bacteriophage, grow it and extract its DNA, sequence the genome, and analyze the sequence relative to other genomes. Significantly more advanced and expensive synthetic biology based high school experiences include Biobuilder™ modules (Kuldell et al., 2015) and summer International Genetically Engineered Machine (iGEM) high school competition (https://igem.org/Competition/High_School). Unfortunately these activities are not very accessible as costs associated with them are in the thousands of dollars, in addition to usually requiring advanced laboratories and more strict biosecurity. To overcome these barriers while preserving aspects of genuine inquiry, some groups have recently allowed students to grow bacteria and decide on which antibiotics to add while following the experiments remotely (Dahan et al., 2019) or in an hermetically sealed system (Fein et al., 2020). Finally a recent project named Biomakerlab is aimed more towards middle school students and emphasizes design in biological systems. Biomakerlab includes 4 modules; making a bacterial fuel cell, using bacterial pigments to design a logo, designing biosensors and using biobuilders' golden yeast (produces β-carotene) to make bread/cakes with students (*BioMaker Lab*, n.d.; Y. Kafai et al., 2017; Y. B. Kafai & Walker, 2020).

4.1.3 Hands-on Molecular biology in informal learning

The previous section focused on formal learning environments, but some have argued that most science learning is done informally (Falk & Dierking, 2010). Informal science learning is usually discussed in terms of places like museums, libraries, nature centers, after-school programs or amateur science clubs (National Research Council, 2010) but reading books and magazines about science and technology, using the internet, and watching science related documentaries and videos were the strongest predictors of self-reported knowledge of science and technology in a survey of Los Angeles residents (Falk & Needham, 2013). Different but related learning goals have been offered for informal science learning (Bell et al., 2012). Many have suggested that emphasis in science learning, especially of the

informal kind, should be placed on meeting people where they are and taking into consideration different 'publics' each with their own social, cultural, and demographic characteristics (Bell et al., 2012; Feinstein et al., 2013; McCallie et al., 2009). Especially considering the rise of the internet, this has caused a shift from pushing for scientific literacy or public understanding of science to a model emphasizing public engagement with science (Block et al., 2016; Kollmann et al., 2012; Stilgoe et al., 2014; K. Todd et al., 2018). Shifting the focus from knowing the textbook to accessing the science you need, from practicing science to judging scientific claims and from positive (or negative) feelings to deep and durable involvement (citizen science) (Bonney et al., 2016; Feinstein et al., 2013; The national science academies of the G7, 2019).

Very little research has been done on so called 'free-choice' adult learning in places like public facing science fairs or events (*BBK Open Science*, n.d.; Block et al., 2016; hackarnaval, n.d.), conferences/exhibitions (*Biodesign Here Now*, n.d., *Fab14 Fabricating Resilience*, n.d., *Global Community Bio Summit*, n.d.) or in community labs (e.g. http://www.hackuarium.ch/en/, https://www.genspace.org/), especially in terms of molecular biology. Some examples include using PCR to detect different PTC (bitter taste receptor) alleles in a university public lab which offers workshops to different audiences (*Génétique, Ateliers Scientifiques, Débats, Dialogue Science Société* | *UNIL* | *Médiation Scientifique*, n.d.), detecting GMOs in food samples with a DIY community (Kuznetsov et al., 2015), or the recent attempt to sequence food products with the public to verify product labeling (Digital Epidemiology Lab, EPFL, n.d.).

In this chapter I will discuss the different formats of workshops conducted with high school students and other diverse audiences and the results of these including feedback by participants (and their teachers) and detailed Pre/Post questionnaires.

4.2 Results

4.2.1 Workshops conducted

After successful pilot workshop/labs conducted in house with different audiences and in different formats (See section **3.2.7**) we scaled up the project to more paticipants. The workshops were done over 25 times in 5 countries (France, UK, Switzerland, USA, Spain), by more than 400 people, half of which were K-14 students in the Paris region, and the other half on diverse groups e.g. researchers, biohackers makers, and the general public (**Table 4.2.1**) And appendix H for representative photos.

Name	Audience	Location	#	Format	Date
High school Marcelin Berthelot	High school students	CRI	4	3x3h	29/11/17
Biodesign class	Design students	CRI	16	3h	01/12/2017
CRI community	Scientists	CRI	7	2h	16/3/18
High school half class	High school students	CRI	9	full day	9/5/18
Hackarnaval	General public	Museum Paris	6	2h	17/5/18
Hackuarium	General public/ DIY	Lausanne	6	3h	1/6/18
FAB14 Solidarity & Ecology	General public	Auray Brittany	20	3h	14-15/7/18
Biodesign Here Now	General public	Opencell London	8	3h	21/9/18
Fete de la science	Children	New CRI	6	15min+5	7/10/18
Global Community Bio Summit	DIYbio	MIT	20	3h	26-28/10/18
Claude Bernard HighSchool	High school students	Paris	36	full day	12/11/2018
Gonnesse	High school students	Ile de France	34	full day	14/11/2018
Saint Germain en laye	High school students	Ile de France	15	full day	15/11/2018
Choisy-le-roy	High school students	Ile de France	34	2h	26/11/2018
Mennecy	High school students	Ile de France	34	full day	03/12/2018
Les Ulis	High school students	Ile de France	52	full day	04/12/2018
La ferté Macé	High school students	Normandy-remote	15	4x2h	12/18
BBK OPEN SCIENCE	General public	Bilbao	20	1h+3h	19/1/19
SDG Summer School	General public	Paris	8	3h	2/7/19
CRI L1	1st year University	CRI	30	3h	27/9/19

Hackuarium	General public	Lausanne	10	1h+3h	5/10/19
Global Community Bio Summit	DIYbio	MIT	30	1h+3h	12/10/19
Etopia Wetlab	Senior citizens	Zaragoza-remote	15	3h	29/11/19
		Total:	**416**		

Table 4.2.1 Details of workshops conducted

4.2.2 Workshop variations and participants

4.2.2.1 High School workshops

Workshops were conducted in French public high schools in the Paris area. Eight classes were chosen through interest shown by the teachers and class staff. Six out of the eight were given the long whole day workshop version (**Table 4.2.2)**. All of these were 15-18 year old students specialising in life and earth sciences (Sciences de la Vie et de la Terre (SVT)).
One other class was younger and did only the 2 hour version (**Table 4.2.3**), while the last class did the workshop by themselves remotely over the course of a few weeks (see Appendix G for detailed fit of workshop to the french curriculum).

Detailed daylong high school workshop workflow:

Time(minutes)	All day format
10	Pre-questionnaire
20	Intro to citizen science, open source, fablabs/making
15	Intro to fluorescence, its uses in biology and how to solder
45	Building and testing the detectors
30	Primer on DNA/Genes/Central dogma/ transgenesis and DNA amplification
30	Going over experiment overview/ importance of controls
Lunch	
15	Going over the protocol again, emphasis on working cleanly and precisely
45	Doing experiment
60	GMO debate+amplification
15	results
15	post questionnaire

Table 4.2.2 Daylong workshop format overview

Workshops were conducted by Tonino Rizo, Guy Aidelberg and Imane Biaz, with support of and in conjunction with the pan european project DITOs (Doing it together science, see: http://www.togetherscience.eu/ and policy brief (DITOs Consortium, 2019)). The working language was mostly French, and usually with one or two of the students' teachers present and more or less active during the day. The days began with the students being given anonymous numbers and then a pre workshop questionnaire (see next section and Appendix F). They were then given a short introduction to collaborative culture: Citizen Science, Open source, FabLabs and making. The second part involved a reminder of light and different wavelengths, the concept of fluorescence and its use in biology, and specifically to detect double stranded DNA, as well as how the detector we will be building works. Next an introduction to soldering was given, mostly emphasizing caution and the high temperature of the soldering iron, and an explanation of which pieces go where, including the polarity of some components (LEDs and capacitors) and the lack thereof of others (resistors and fuse). This introduction took about an hour, then groups of 2-3 students were given a soldering iron and solder, along with all the components to construct the detector. They were given another hour to actually build it, almost all groups successfully assembled and connected the detector with some groups needing a bit of help to correctly place and solder some components or remove excess solder which caused a short circuit. Both we and the teachers clearly saw great teamwork and cooperation during this part; One student needed to hold the PCB and parts while another student soldered them together, this created trust and concentration. For many of the students this was the first time they actually built an electrical device that worked and was useful, and in general when they saw their detector light up it caused great happiness and excitement.

Following a short break came an hourlong theoretical part. This began with a short TED-ED video (https://www.youtube.com/watch?v=z9HIYjRRaDE) on where genes come from, and how they change and evolve over time. Focusing in on the important parts, that each cell has chromosomes, which are made of DNA, which is a long chain of complementary bases. Some parts of these chromosomes code for specific

genes, which can be expressed and can have a phenotype, including a discussion of the central dogma. We then discussed transgenesis, GFP and specifically the P35 promoter found in most commercial transgenic food crops. We then asked how do we detect this specific marker? This led to a reminder on DNA replication in cells which leads to in vitro DNA amplification, including primers and their specificity which can lead to exponential amplification. Then detection of the specific DNA with the aid of fluorescence. We then briefly went over the experiment:

The 2 different samples: we give participants a sample with a letter (which can have either 0% 0.1% 0.5% 1% 5% or 10% w/w GMO inside, this is double blinded, i.e both the participants and the instructors do not know which sample is which) used to benchmark the method in laypeople's hands (see section 4.2.3). And the other sample that participants brought with them to check for GMOs, or common foods with and without are also available. The simple DNA extraction, smashing adding water and then boiling. The main question, does the sample have the marker we are looking for?
Adding the extracts to the mastermix and what it contains. Then incubate and observe the results. In preliminary tests we found one of the most difficult and important things students can learn from this experiment is on the use of controls both positive and negative which we need in order to have any conclusions about our experiments. We discuss these controls in our context, what are the positive and negative controls here and the plant DNA experiments to control for DNA extraction. The students then go to lunch.

After lunch we went over the protocol again, especially possible failure points such as how small the piece of food should be, caution with the boiling water, what exactly we put in each tube, how to use a micropipette, as well as working cleanly without contaminating the samples, changing tips and marking the tubes and caps clearly so they can be identified. Students were then handed protocols and all the necessary materials to do the experiment. Conducting the experiment usually took less than an hour. Facilitators walked around the class answering any questions and trying to make sure students were following the protocol.

When all the students were finished with the protocol their PCR tube strips were collected and placed at 63℃ for an hour. During this hour, two different discussion methodologies were tried, the first was an open discussion on GMOs answering students questions and discussing agriculture and the use of GMOs today and in the future, how everything we eat has diverged from their ancestors, traditional breeding vs mutagenesis vs RNA interference vs transgenics and CRISPR. We also talked about other uses such as bioproduction of insulin and chymosin, cancer immunotherapy and humanised pigs for organ transplant.

In the other version students were placed into around 8 different groups and randomly assigned to research and then defend/debate a specific point of view regarding GMOs (Goldschmidt et al., 2016). These groups could be broadly categorized into 5: Politicians, Doctors, Farmers/agronomists, Citizens and Researchers, these can then be further subdivided into local and (inter)national politicians, family and/or research/cancer doctors, organic small producers and large scale farmers, etc. Students were given 15-20 minutes to look online for views and information on the subject from the point of view they were assigned and then a half an hour debate was started and moderated by one of the teachers or researchers. This seemed to work better and allowed the students to express themselves, be active instead of passive and to see the issue from many different points of view. Students were highly engaged and passionate and seemed to really enjoy thoughtfully taking on different roles.

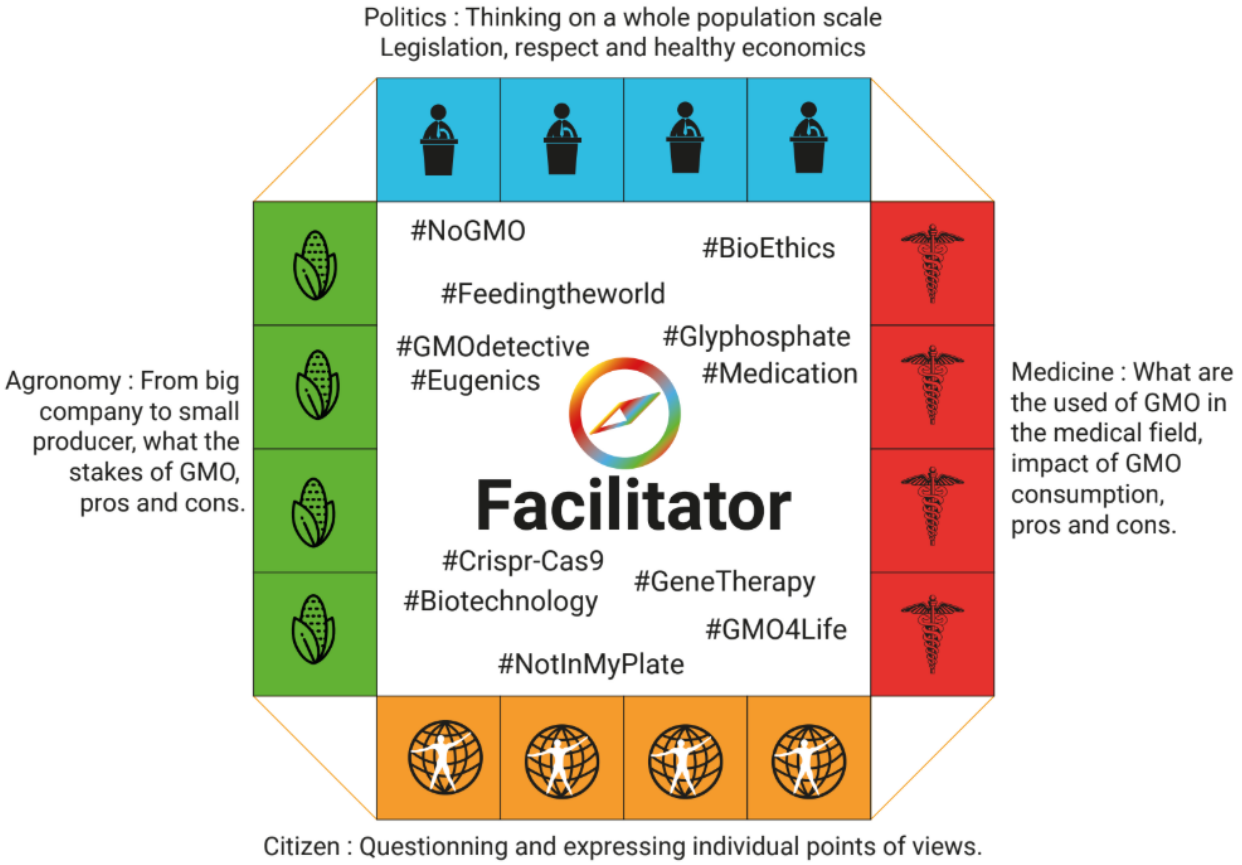


Figure 4.2.1 Role playing debate schematic

After the discussion/debate the PCR tubes were taken out of incubation, allowed to cool and then the results analyzed by the students. Letting them think for themselves what the results mean, and then guiding them if needed using the following scheme (See also appendix E):

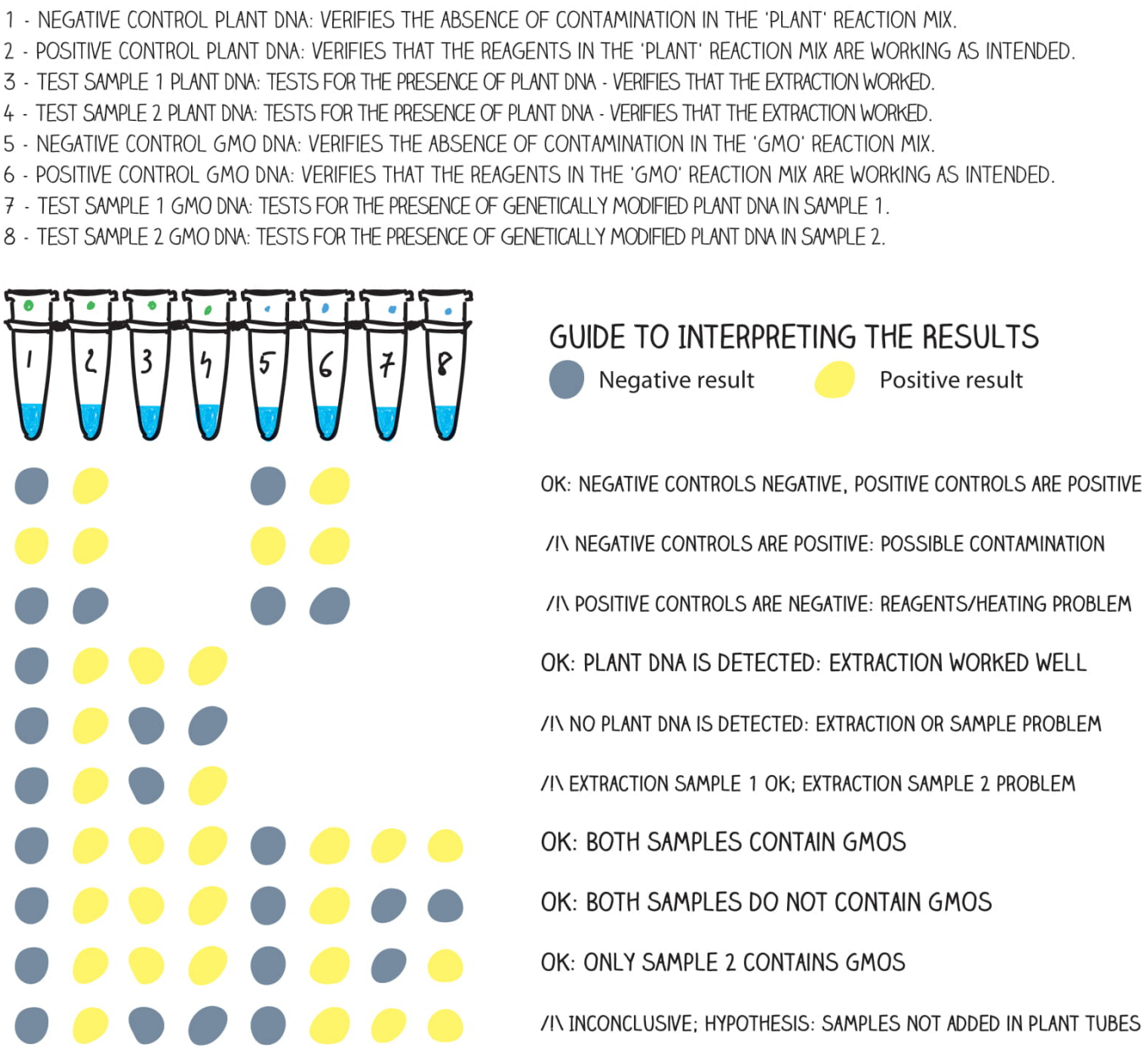


Figure 4.2.2 Guide to results interpretation.

After analysis, interpretation and recording of the results students were given a similar but longer post workshop questionnaire (see section **4.2.4** and Appendix F).

4.2.2.2 Other workshop versions

Most other workshops were of a shorter 3 hour format (**Table 4.2.2**) similar to the what is described in the previous chapter (**3.2.7**), some of these were supplemented

with another one hour session, where participants built their own detectors (**3.2.3**). Public workshops began by asking participants who they were and why they were interested, and different aspects were emphasized accordingly, for example DIYbiology participants were more interested in technical details of LAMP, while the general public was usually more interested in GMOs. A two hour version usually meant shortening the debate/discussion part and analyzing the results later (in the day or week).

Time (minutes)	(Optional 1h making detector)+3h format
15	Introduction and Pre-questionnaire
15	Intro to fluorescence, its uses in biology and how to solder (Optional)
45	Building and testing the detectors (Optional)
20	Primer on DNA/Genes/Central dogma/transgenesis and DNA amplification (emphasis depends on audience)
10	Going over Experiment overview, importance of controls/pipetting
40-50	Experiment
60	Discussion+amplification
15	Results
15	Post questionnaire

Table 4.2.3 Short workshop format overview

Undergraduate students did the 3 hour version but with a debate (**Fig 4.2.1**), with other audiences the hour of incubation time was used differently, usually discussion on GMOs in general, very much dependent on participant led inquiry.

4.2.3 Assay results in laypeople's hands

In the experiment, we gave participants a sample with a number or letter (which had either 0% 0.1% 0.5% 1% 5% or 10% w/w GMO soybean or corn inside, this was set up as a double-blind experiment, i.e. both the participants and the workshop facilitators do not know which sample is which) it was used to benchmark the method in laypeople's hands. Results given here (**Fig 4.2.3**) are for the public participants and 1st year undergraduate students, half of which used a “wet" version, and the other half the optimized freeze-dried version (High-school results are discussed later in section 4.2.6). Common measures of diagnostics include (Šimundić, 2009)

Sensitivity=true positives/(true positives+false negatives)= 53/(53+3)=95% in our case. Sensitivity=true negative/(true negatives+false positives)=11/(11+1)=92%. And effectiveness or diagnostic accuracy=(true positive+true negative)/(all)=(53+11)/(53+11+3+1)=94% , which asses the overall percentage of correct results. This is was an encouraging result, especially given that most of the participants had never used a micropipette before, and points to the robustness and ease of use of the developed methodology.

Percent GMO	0%	0.50%	1%	5%	10%	100%	Overall correct
Positive/ All	1/12	3/4	17/18	4/4	17/18	12/12	64/68 94%

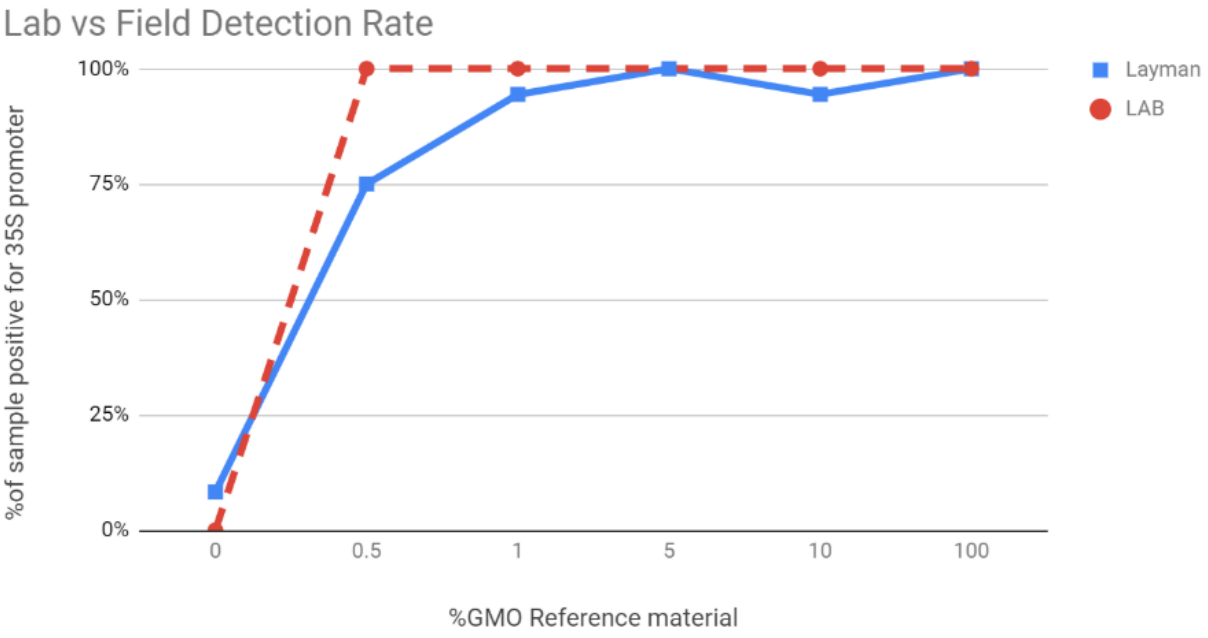


Figure 4.2.3 Detection rate of blindsamples by lay people using GMO Detective

4.2.4 Questionnaires

Pre and post workshop questionnaires were developed (as well as teacher feedback forms) to help assess the workshops. Identical questions were asked both pre and post workshop about participant knowledge/confidence, interest and attitudes. Additional post only questions included feedback on what was difficult, skill mastery and more general feedback on understanding and empowerment. Questions were loosely based on (Bandura, 2006; Dawson & Schibeci, 2003; A. Huang, 2019), with adaptations to our settings, and except for general demographic data (age, gender, etc.) and some open ended questions at the end, responses were given on a five point Likert-type scale.

Prior to use, several of our peers and two high school teachers reviewed the questionnaires to assess readability, appropriate knowledge level questions, and overall understandability. After the first high school class (data omitted) some small changes were made, such as adding a sixth option of “I don't know” or “I knew this before” to some of the question sets (see Appendix F). Obtaining responses to the post workshop questionnaire in many informal settings was found to be quite difficult as often there were often many other activities going on and a large proportion of the participants left right after looking at their results.

4.2.4.1 Improved self reported understanding of key concepts

The first set of questions were on the self reported understanding of key related concepts, and if participants can explain the concept to a peer: The structure of DNA? What is a gene? What is a GMO? DNA replication? DNA amplification techniques? Fluorescence? Wavelength of light? And Citizen Science.

We found significant improvement in self reported ability to explain all concepts, especially for those introduced in the workshop (e.g. those where little was known to the students about the topic before see: **Fig 4.2.4**). We found a rise of at least 20% in the ratio of students answering yes or perfectly (4 or 5), after the workshop

compared to before the workshop on all concepts. An especially large risewas found in the concepts central to the workshop: What is a GMO increased over 85% (from ~50% before while after more than ~95% answer yes or perfectly). On DNA amplification techniques understanding increased over four fold. With the largest difference in the concepts of fluorescence and citizen science, where self reported knowledge increased almost 6 and 27 fold respectively. Additionally over 94% of all participants answered more or less, yes or perfectly (3,4 and 5) to being able to explain all the concepts after the workshop compared to 63% before the workshop, a 50% increase (**Fig 4.2.5 A**).

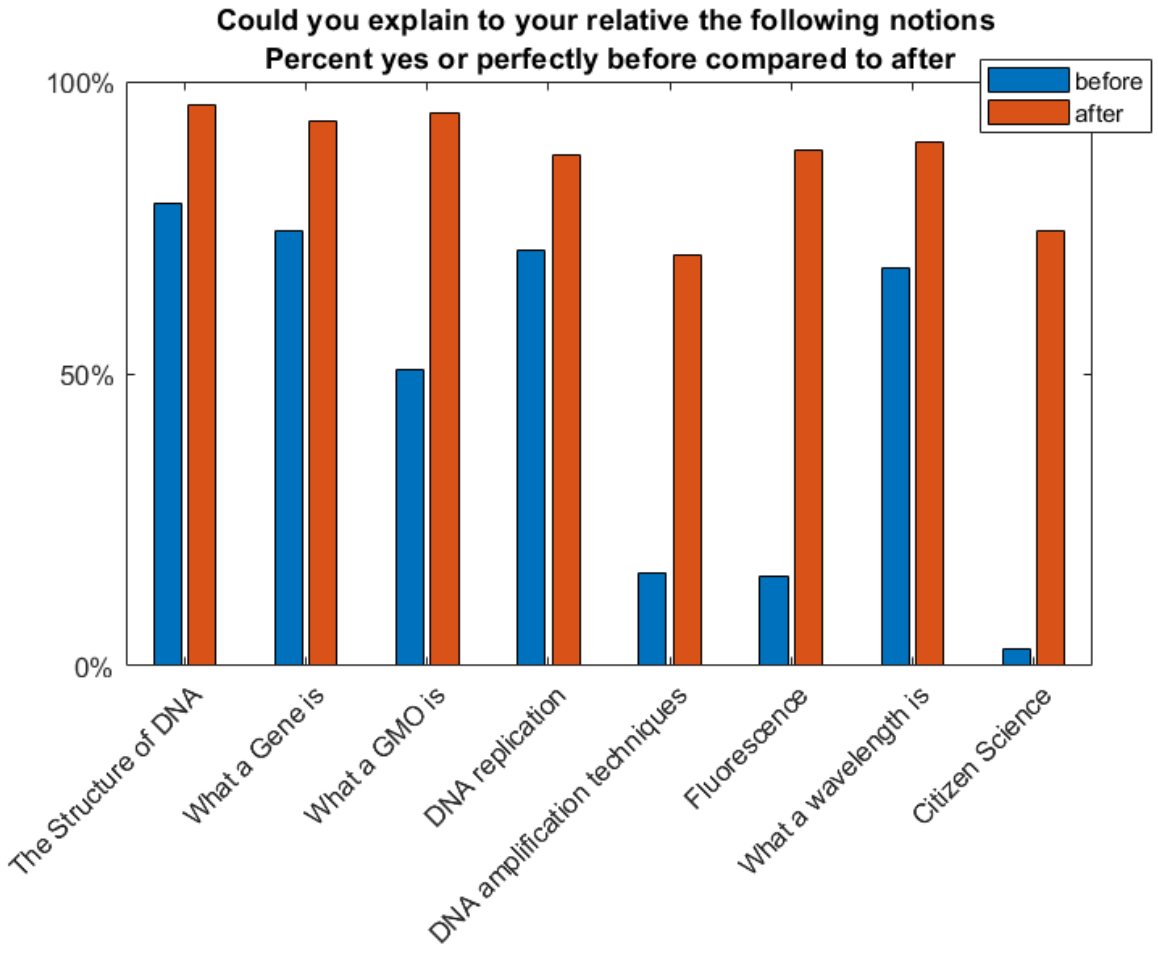


Figure 4.2.4: Workshop increased students' understanding of key concepts.
High-School students self reported understanding/knowledge (confidence in ability to explain) of key concepts grows after workshop participation **N=146**. Those answering 'yes' and 'perfectly' were binned together.

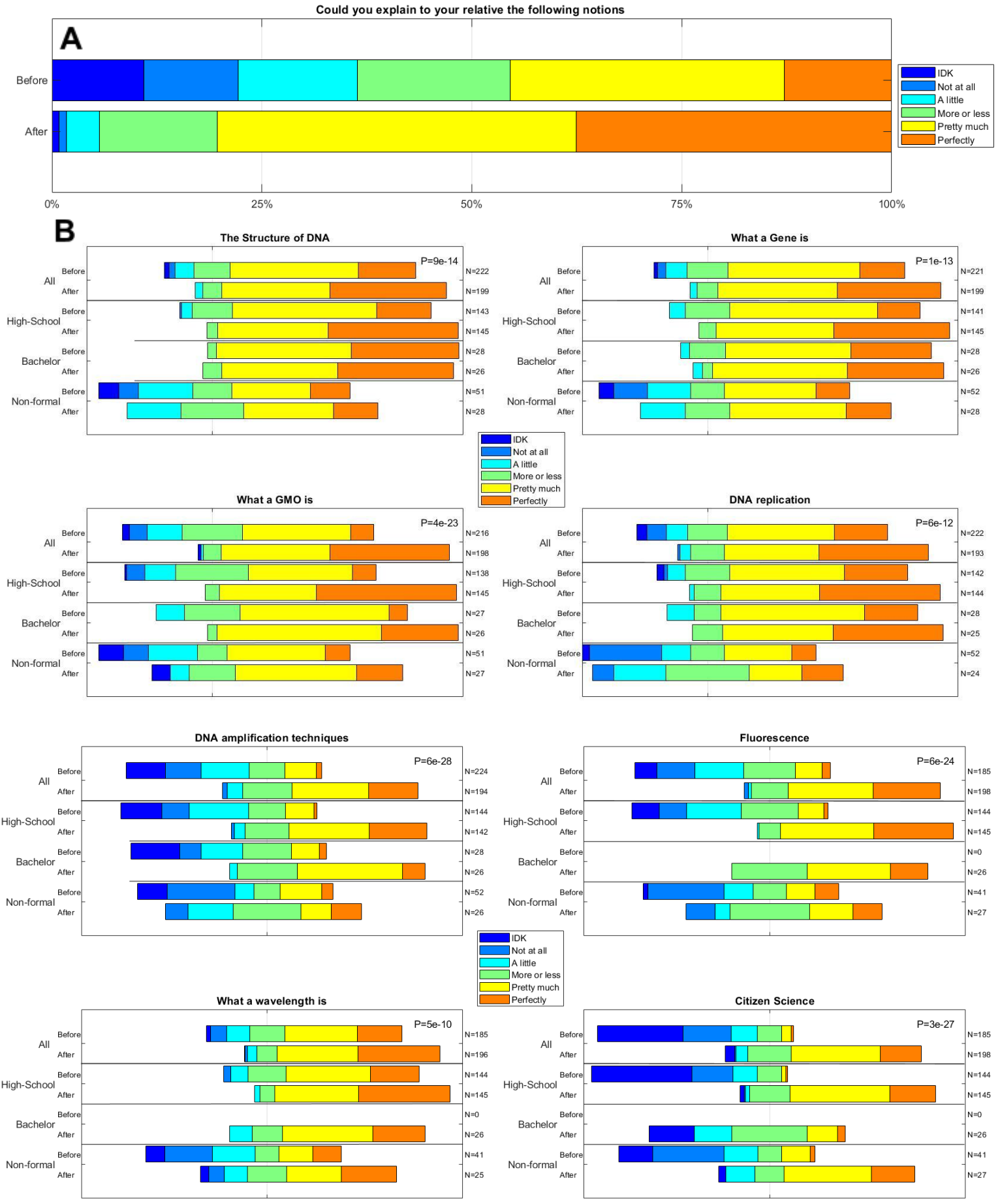

Could you explain to your relative the following notions
A
Before
After
0%
25%
50%
75%
100%
IDK
Not at all
A little
More or less
Pretty much
Perfectly
B
The Structure of DNA
P=9e-14
What a Gene is
P=1e-13
What a GMO is
P=4e-23
DNA replication
P=6e-12
DNA amplification techniques
P=6e-28
Fluorescence
P=6e-24
What a wavelength is
P=5e-10
Citizen Science
P=3e-27
All
High-School
Bachelor
Non-formal

Figure 4.2.5 Detailed responses and statistical significance of all participants' answers on self reported understanding of key concepts. **A)** Aggregate of all participants and all questions. **B)** Responses to each "can you explain to a peer/relative" question, both before and after the workshop of all participants, along with a breakdown of the different groups (High-school, Bachelor and Non-formal) with number of respondents to the right. IDK corresponds to "I don't know". P-value of a Wilcoxon signed rank 2-sided test for paired responses.

By breaking the responses down into the different groups (High-school, Bachelor and Non-formal), some other general observations can be made. The results of the high school and bachelor students are very similar, with the bachelor students giving slightly more confident answers on the first 5 DNA related questions before the workshop (**Fig 4.2.5 B**). The improvement is greater in the high school students compared to the bachelor. This is probably due to the shorter length of the bachelor workshop, along with the higher level of background knowledge the bachelor students at least believed they had. Non formal participants in general reported lower understanding, especially before the workshops, compared to students, except in DNA amplification techniques, fluorescence, and citizen Science. This probably has to do with some of the participants being so-called "biohackers", which are more familiar with these concepts (**Fig 4.2.5**).

4.2.4.2 Increased self reported interest level in related fields

We next asked about participants on their interest level in related fields. Science, Biology, Electronics, Physics, Citizen Science, DIY, Open source, and Fablabs. We found that the interest of high school students rose in all related subjects, especially those they did not know about before (**Fig 4.2.6**). For example, before the workshop, only ~6% of students answered that they were interested or very interested in open source, while after more than 50% did, an 8 fold increase. An even larger change was seen with interest in Fablabs, rising more than 23 fold. Slightly smaller changes were seen in changes regarding the citizen science and do it yourself fields, where interest rose 467% and 83% respectively. In the other fields we see a much smaller change as the students were already very interested in the subject before the workshop (**Fig 4.2.6**).

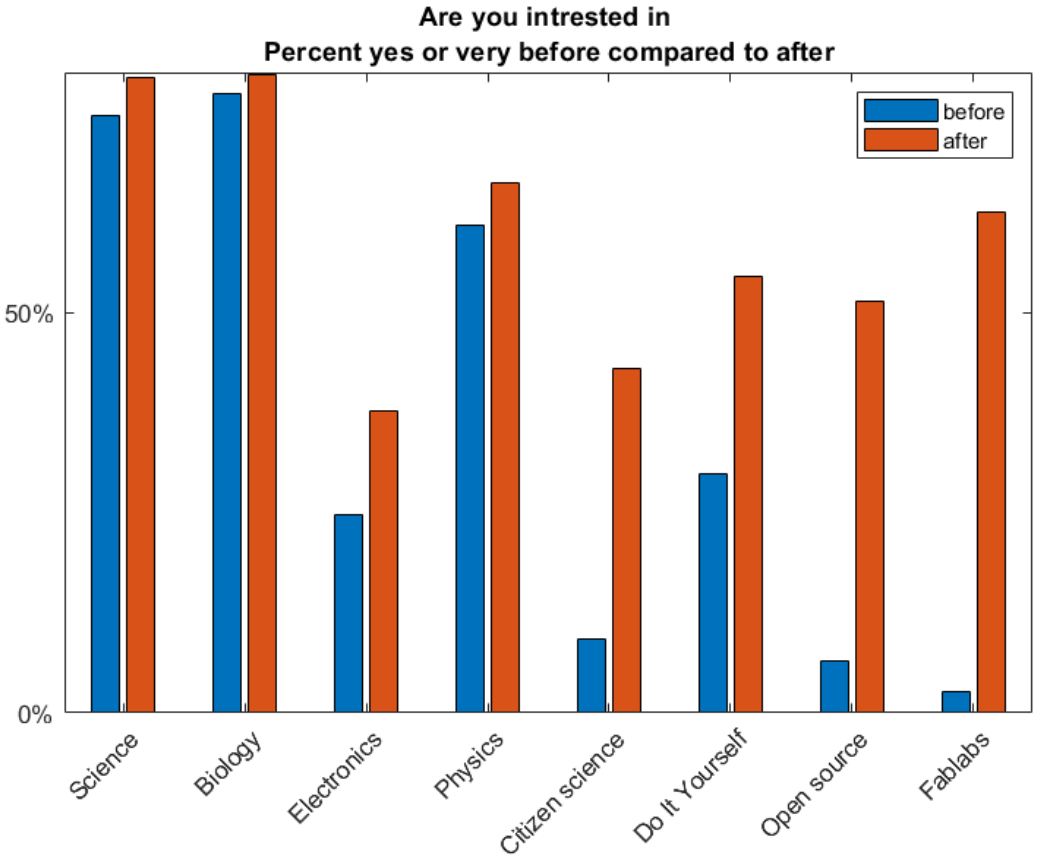


Figure 4.2.6: Interest increases in all related fields, especially for those which they knew little about before. High-School students self reported interest in related fields before and after workshop participation, N=146.

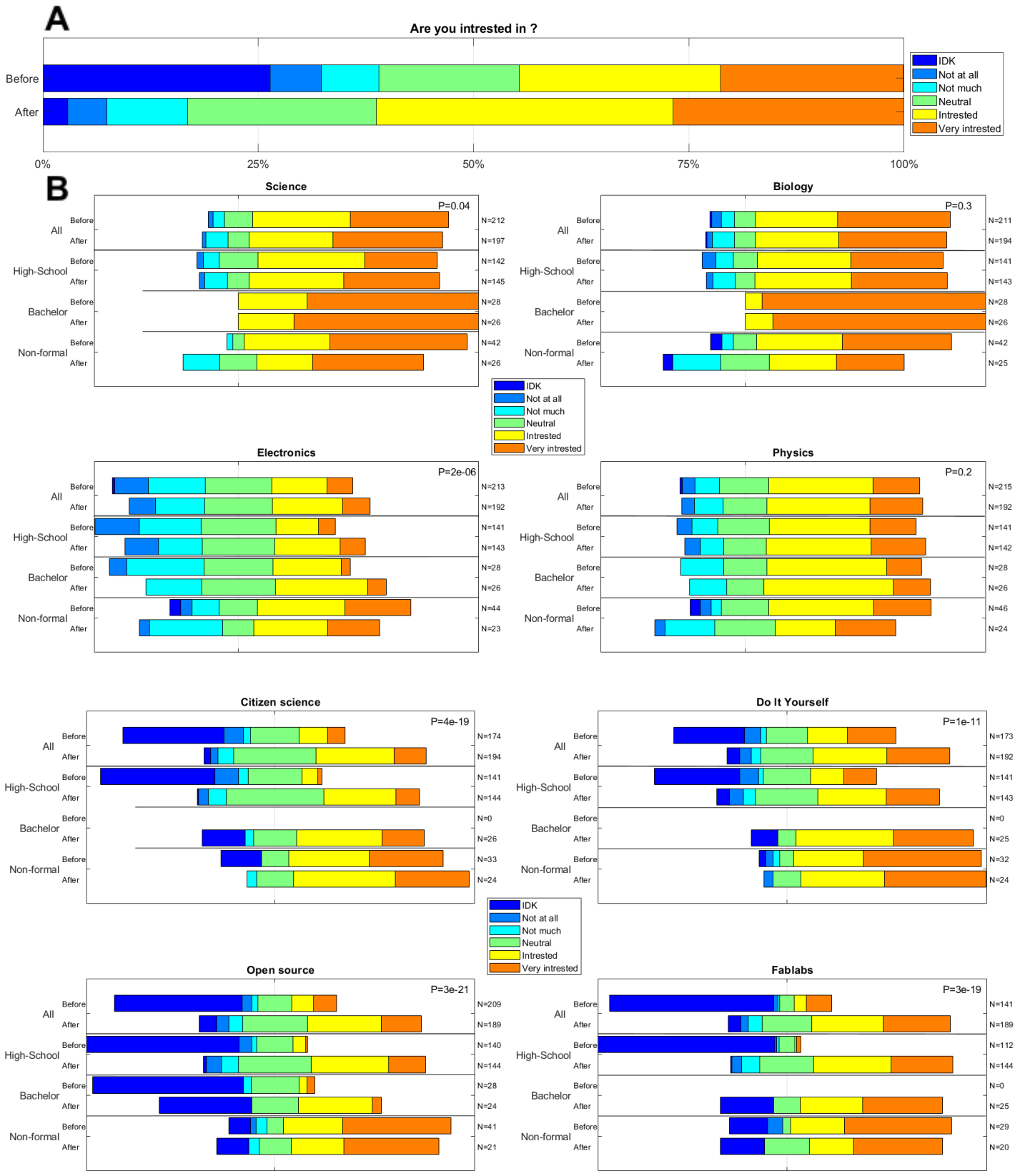

A
Are you intrested in ?
Before
After
0%
25%
50%
75%
100%
IDK
Not at all
Not much
Neutral
Intrested
Very intrested
B
Science
P=0.04
Biology
P=0.3
Electronics
P=2e-06
Physics
P=0.2
Citizen science
P=4e-19
Do It Yourself
P=1e-11
Open source
P=3e-21
Fablabs
P=3e-19
All
High-School
Bachelor
Non-formal
Before
After

Figure 4.2.7 Detailed responses and statistical significance of all participants' answers on self reported interest level in related fields. **A)** Aggregate of all participants and all questions. **B)** Responses to each "are you interested in" question, both before and after the workshop of all participants, along with a breakdown of the different groups (High-school, Bachelor and Non-formal). IDK corresponds to "I don't know". Number of respondents to the right. P-value of a Wilcoxon signed rank 2-sided test for paired responses.

Interest in general rose significantly, before the workshop 42% of respondents were interested or very interested in a subject, while after the workshop over 62% were (**Fig 4.2.7 A**). Again breaking the responses down into the different groups (High-school, Bachelor and Non-formal), some other general observations can be made. Bachelor students were much more interested in science and biology, as they had just started a 3 year degree in these subjects. While non-formal participants were much more interested and knew more about the fields of Electronics, Citizen Science, DIY, Open source, and Fablabs, especially before the workshops as these were often conducted in or with biohacker and maker spaces/communities (**Fig 4.2.7 B**).

We additionally asked not only if students were **passively** interested but if they were willing to **actively** invest their time to know more (go deeper) about these same specific subjects. We found a significant rise in the number of students willing to actively invest time in all the related subjects, except for Physics which was not really discussed, this serves as an internal control, showing that the sharp rises in all other fields can be attributed to our intervention (**Fig 4.2.8**).

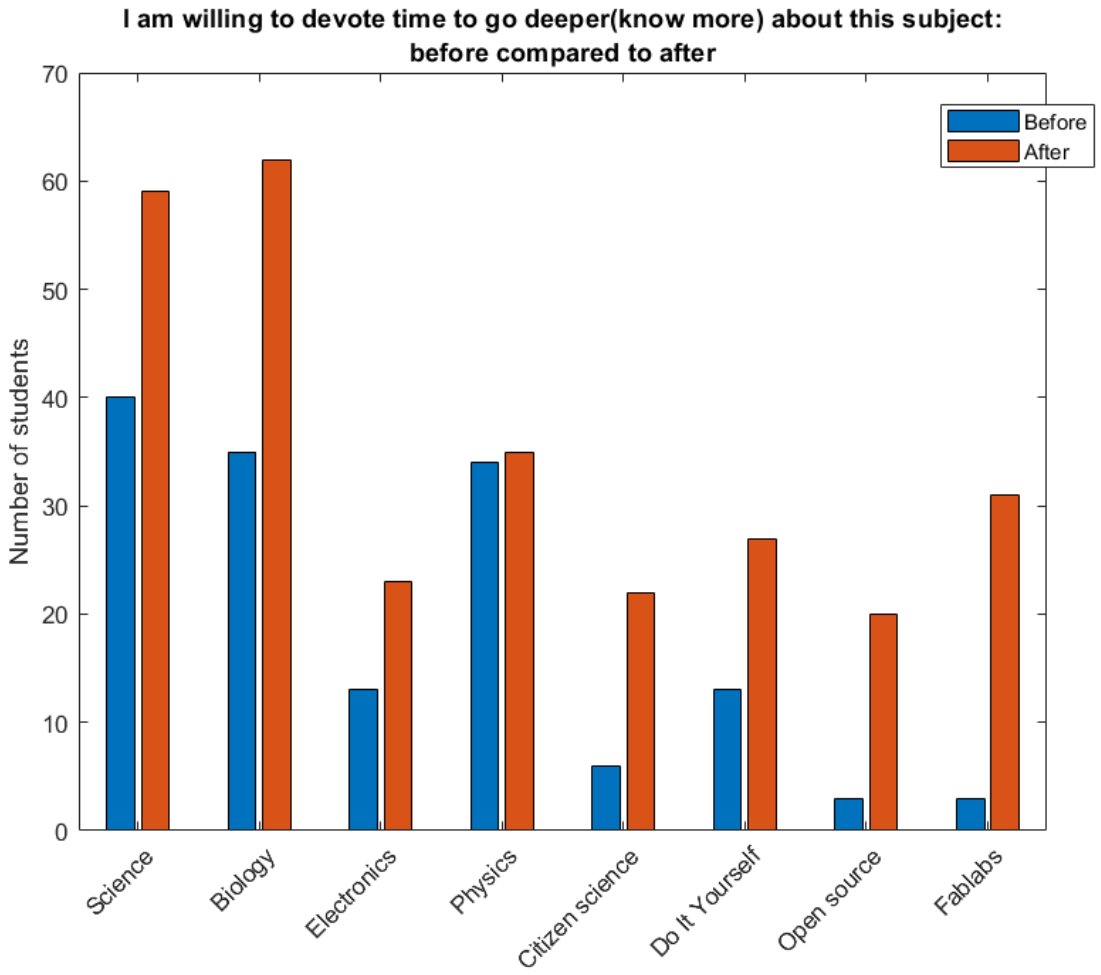
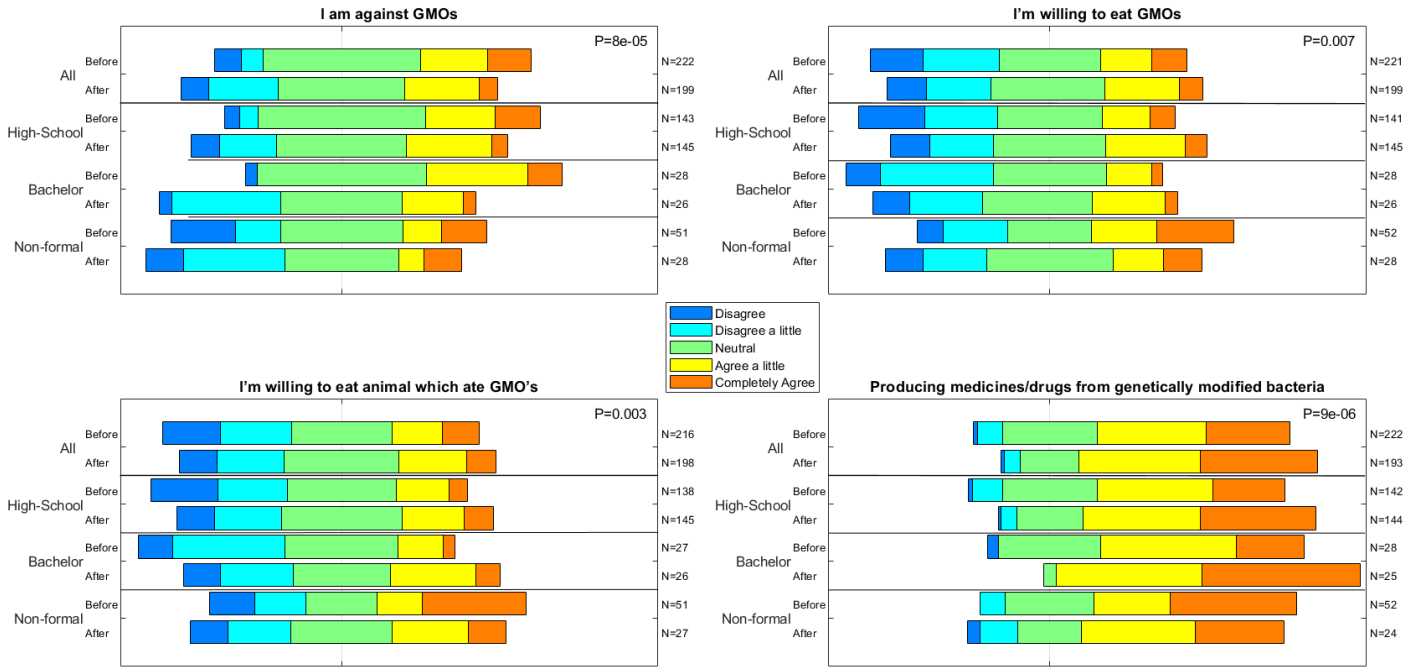


Figure 4.2.8: Increases in number of students willing to actively invest time in related subjects. Number of High-School students self reported engagement/ willingness to invest time in related fields before and after workshop participation, N=146.

4.2.4.3 Modest changes in self reported attitudes towards genetic modifications

We next asked about participants' attitudes towards biotechnology. We do not observe large changes in views regarding GMOs (**Fig 4.2.9**). No change was expected here, as we did not try to convince or change students' minds actively. Depending on the context and questions some examples were given during the workshop, these include modifying crops for increased resistances to pesticide/insecticide/drought, using bacteria to produce insulin, artemisinin and

chymosin for diabetics, malaria medication and cheese production respectively. Other examples of current uses in medicine included cancer immunotherapy, where immune cells are reprogrammed to target cancer, and ongoing work on humanizing pigs, for organ transplants. Student results seem in concordance with findings by (Klop & Severiens, 2007), on Dutch 16 year olds, which identified four main groups labeled : 'confident supporter' (22%), 'not sure' (42%), 'concerned sceptic' (18%) and 'not for me' (17%). The non-formal participants were more diverse in their opinions (**Fig 4.2.9**) and less against GMOs, again probably due to the 'biohackers' participation. During the debate/discussion part in non-formal workshops, if I gave an opinion it was usually from a devil's advocate stance simply trying to show participants other points of view. Most respondents seemed to find the use of GMOs for medicinal or environmental uses agreeable. Very significant changes from before to after were only observed for the questions about being against GMOs, with less participants agreeing after the workshop (35% compared to 28% after), and for producing medicines/drugs from genetically modified bacteria, where more participants agreed to this proposition after the workshop (61% before compared to 75% after) (**Fig 4.2.9**).



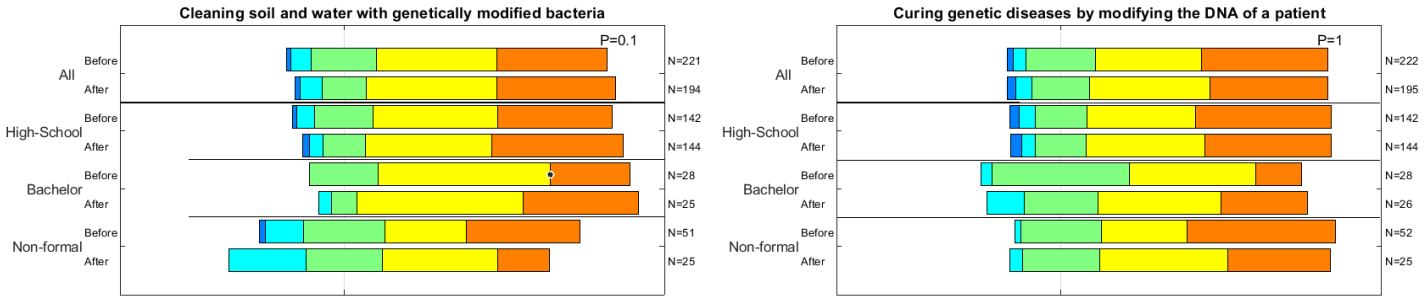


Figure 4.2.9 Detailed responses and statistical significance of all participants' answers on attitudes toward genetic modification and its uses. Responses to each question, both before and after the workshop of all participants, along with a breakdown of the different groups (High-school, Bachelor and Non-formal). Number of respondents to the right. P-value of a Wilcoxon signed rank 2-sided test for paired responses.

4.2.4.4 Ranking and probing the difficulty of different notions

We also asked about the difficulty of understanding some of the discussed concepts. All concepts were rated as easy or very easy to understand by a majority of the participants, except for DNA amplification techniques, which was the most difficult to understand (**Fig 4.2.10**). This was covered quite briefly and is not usually covered in school curriculum or in popular media. LAMP was not discussed with the students, a simplified version of PCR was explained, and we told them we do something similar at a constant temperature. In some of the non-formal settings it was discussed much more in depth, depending on participants' level of interest. More emphasis was later placed to explain the amplification, also the wording here might have not been perfect as only one or maybe 2 techniques were introduced, compared to the other concepts which are more straightforward and narrow.

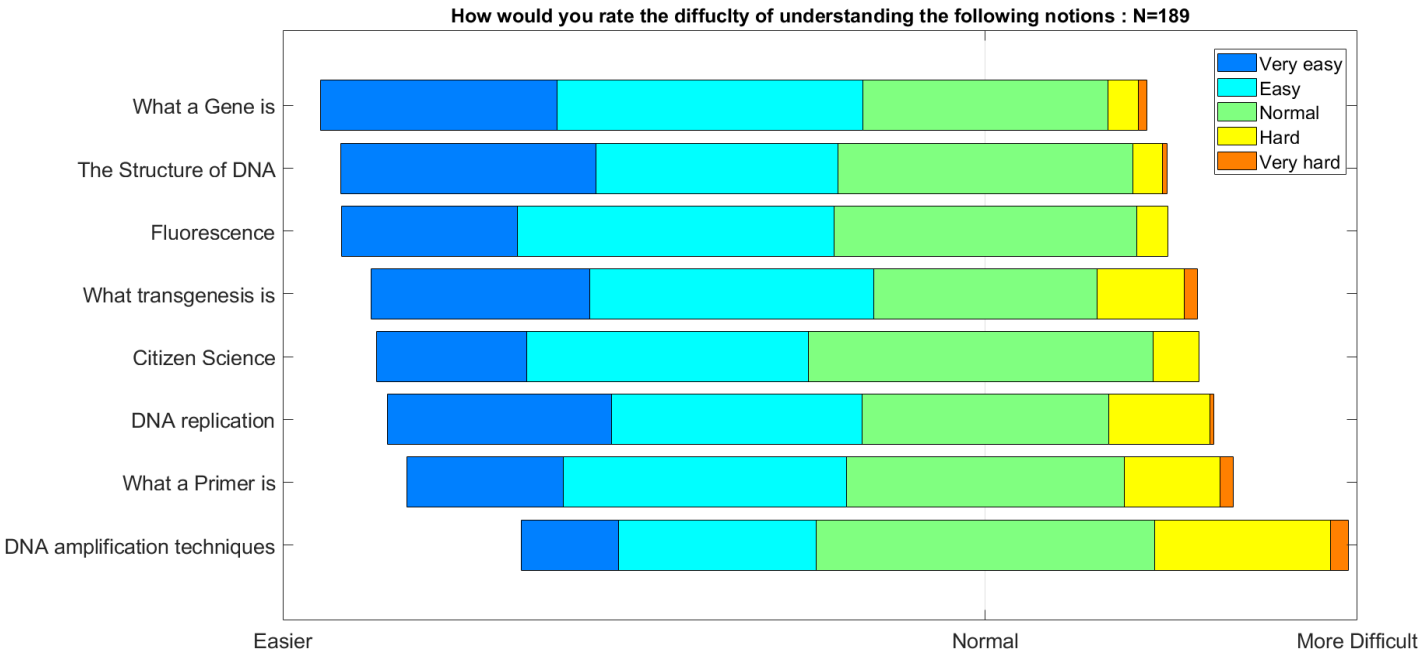


Figure 4.2.10 A majority found most of the concepts easy to understand. All participants' responses on the perceived difficulty level of different key concepts/ notions, sorted from easiest on top to most difficult on the bottom.

4.2.4.5 Self reported mastery of fundamental skills.

When participants were asked if they mastered the skills relevant to the workshop, the vast majority answered "yes" or "completely" (**Fig 4.2.11**). The highest ratings were given to using a micropipette and working in a group (over 90% yes or completely). This is encouraging for a few reasons. First a significant emphasis was placed on pipetting, as this was found in the preliminary workshops to be the most difficult part for lay people/students, but also what made them feel most like "real" scientists. Pipetting was first tried out on only water before pipetting the reagents themselves, and attention was given such that all members of a group participated, and pipetted parts of the experiment. This is also one of the most important basic skills in biology experimentation, which can then be used in many other contexts. It is also surprising as less than 20% of participants replied that they knew this before the workshop, whereas over 35% said they knew how to work in a group before (**Fig 4.2.11**). Slightly lower mastery was reported for formulating ideas, conducting a scientific experiment, reflecting on a socio-scientific subject with a critical eye, as well as interdisciplinarity and taking part in a citizen science project, in that order.

Only 3% said they knew before how to take part in a citizen science project, whereas almost 70% replied “yes” or “completely” after, which is a promising result. Mastery of generating and analysing data was reported as the lowest, with only 66% replying yes or completely, implying more emphasis should be placed here to further empower participants, or again separate this category into 2 different ones might give more precise answers regarding which part was more difficult (less easy to master) generating or analysing data (**Fig 4.2.11**).

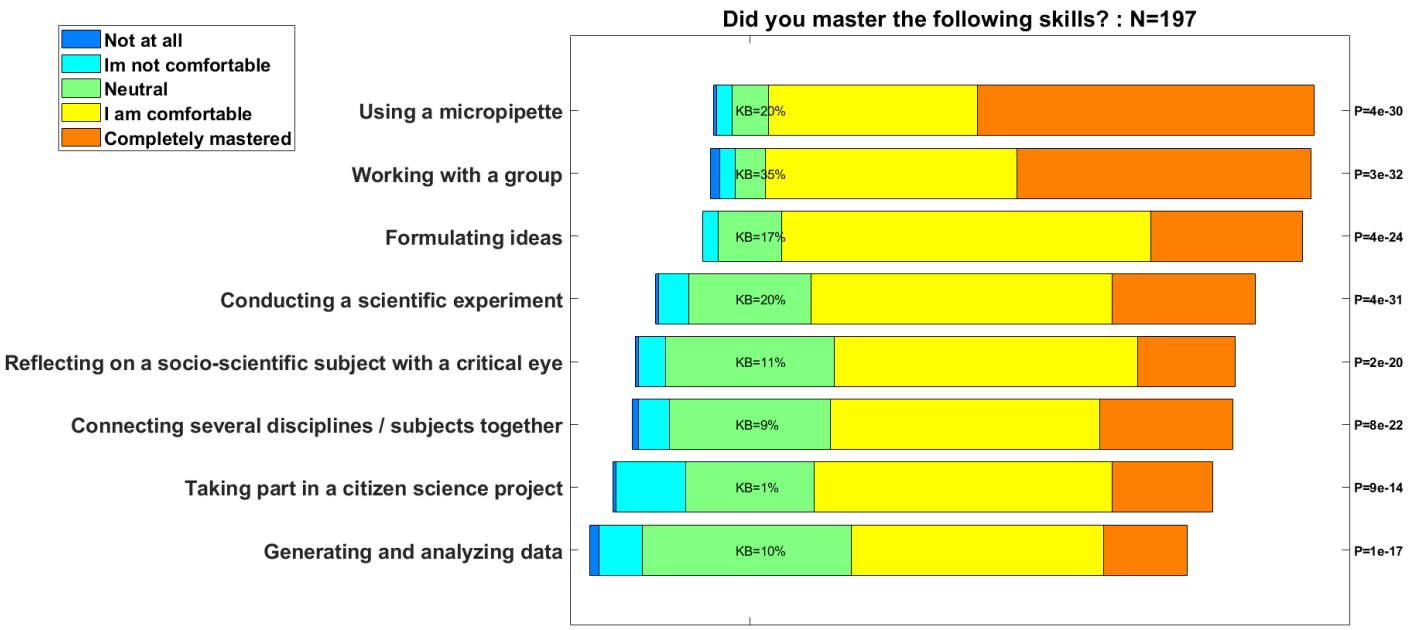


Figure 4.2.11 Most participants feel comfortable or that they completely mastered all relevant skills. All participants responses on self reported mastery of skills used during the workshop. Arranged by reported mastery. P-value for deviation from neutral (Wilcoxon test). KB-percentage of participants answering I knew this before.

4.2.4.6 Overall workshop outcome assessment

Finally a series of more general agree/disagree statements were asked which aimed to assess if participants

1) Found the experiment difficult to do, understand or interpret

2) Could explain what is in each of the tubes, what is a positive and negative control, and if they could repeat the experiment with somebody else.

3) General assessment: This workshop made me want to do some research; I know what a Fablab is and what can be done there; I had a good time.

4) Empowerment, which was the main feeling/attitude we hoped to give to participants. These include: I discovered that there are plenty of resources at my disposal to conduct my own actions. I feel comfortable with the realization of a scientific experiment. I learned the importance of sharing knowledge. I learned that I could take part in scientific research. I was interested in what I did and learned.

Most of the participants found the experiment easy to do, understand and even to interpret the results (67%, 64% and 55% respectively answered “disagree” to finding them hard) (**Fig 4.2.12**). This is promising as a large part of the work was trying to make the entire experience as simple and clear as possible. Interpreting the results seemed to be the most difficult part, this could be due to a few factors, such as ambiguous results (see section 4.2.6), a limited number of USB cables/detectors, participants pushing and shoving excitedly to see their results, and the limited amount of time/facilitators to help explain/guide the interpretation. More emphasis will be placed here going forward, including giving out an interpretation and analysis sheet (See Appendix E and **Fig 4.2.2**).
Only 6-7% of participants disagreed that they could explain what is in each tube and what are positive/negative controls or that they could repeat the experiment. The vast majority agreed with these statements (70-74%) (**Fig 4.2.12**). These results demonstrate the efficacy of our approach, as most of the students did not seem very familiar with the concept of controls during the workshop, and it appears that our explanations and examples at least gave them confidence and an introduction to this important experimental concept. An even higher agreement was reported on the topic of Fablabs, in contrast to the reported interest before the workshop which was very low (See **Figs 4.2.6 and 4.2.7**). This result along with the even higher agreement with the four following statements (scientific experiment, plenty of resources, sharing knowledge and taking part in research) indicate we succeeded to empower participants, giving them confidence as well as introducing and making available a variety of tools, skills and resources. Finally the highest agreement was with the statements on being interested in what they did and learned and having a good time (93% agree or completely agree) (**Fig 4.2.12**).

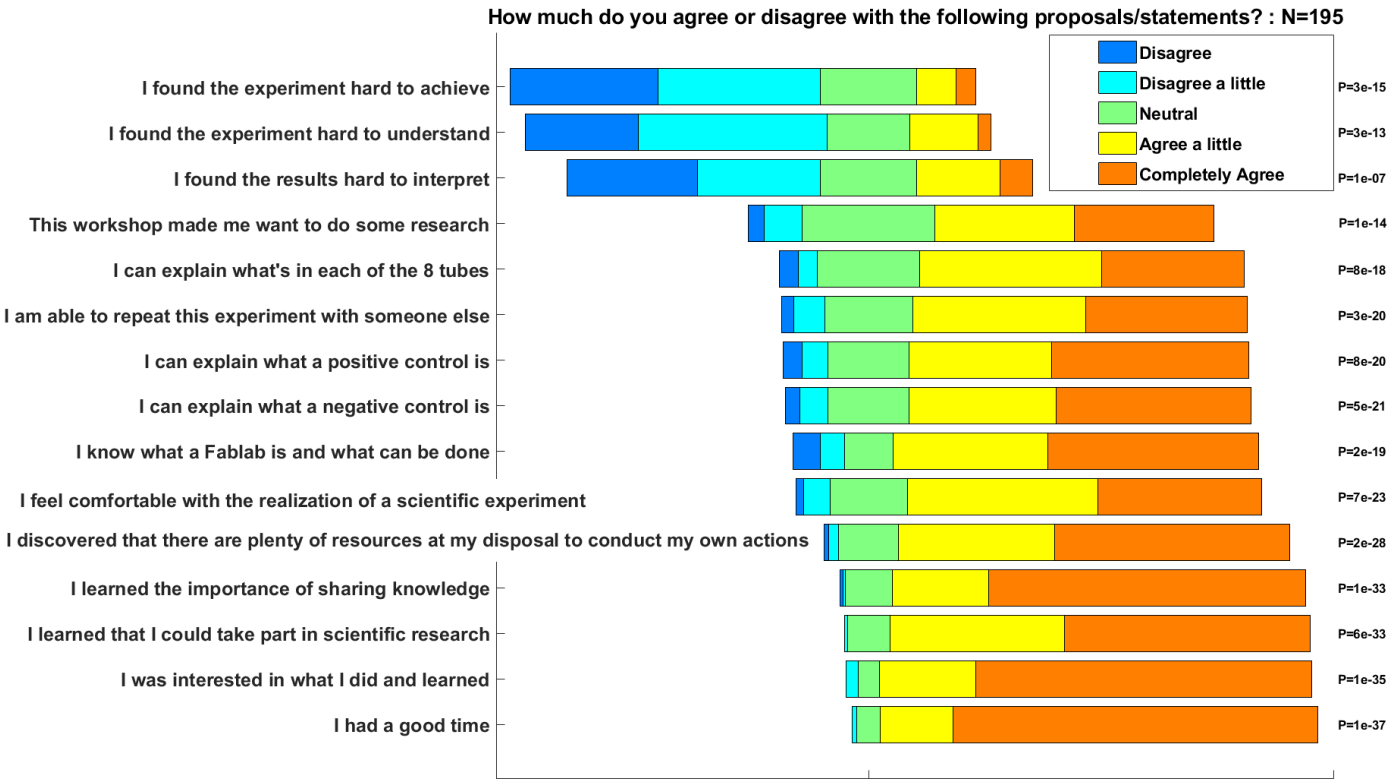


Figure 4.2.12 The workshop is simple, empowering, educational, interesting and enjoyable. All participants' responses and agreement values on a variety of agree/disagree statements. Sorted by level of agreement. P-value for deviation from neutral (Wilcoxon test).

4.2.5 Teachers' feedback on the workshop

Long and more open ended questionnaires were also sent to the teachers who accompanied the students in the formal settings, around two weeks after the workshops took place. Teachers were more or less involved, and mostly took a back seat approach, helping where they could, and taking on a more disciplinary (e.g. telling students to be quiet if they were interrupting) than an active lecturing role. In general the teachers were very enthusiastic/positive in their responses.
All teachers answered yes to the Yes/No questions: Would you recommend this workshop to a colleague? Would you be willing to participate again in this program next year? Would you be willing to support the commercialization of this as an educational kit? And 5/7 agreed they would be interested in co-writing / testifying in a possible article.

On a scale of 1 to 5:

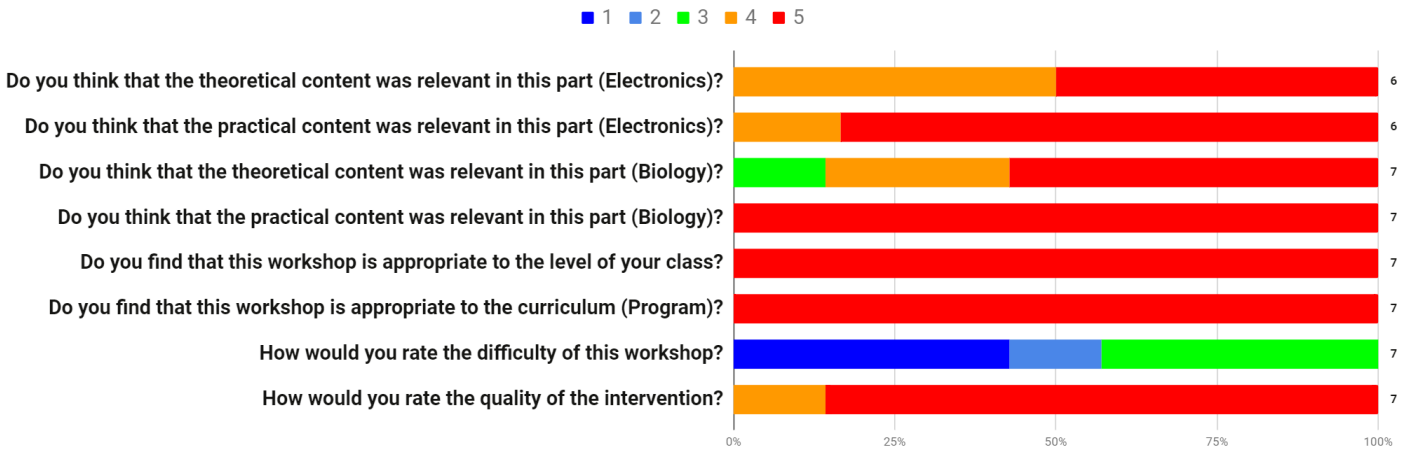


Figure 4.2.13 Enthusiasm of classroom teachers. Teachers of participating classes' responses to Likert-like (1-5) questions. Colors correspond to answers, number of respondents on the right (One class did not solder the the detectors, i.e. the electronics part)

Teachers were particularly positive in regards to the practical aspects of the workshop, both the biological and electronics parts (**Fig 4.2.13**). The theoretical part was rated slightly lower but still high, with all 4-5s except for one 3 for the biological part given by the undergraduate level teacher, this was a short workshop (three hours) and the theory presented was just enough to make sure everyone was on a level field, a mix of the high school theory and what is given to the general public. Also less was known about what they already knew. This could easily be improved by working more closely with university level instructors.

The highest scores were given to the questions on appropriateness to the level of your class and fit to the curriculum (**Fig 4.2.13**). This is strongly supportive of our goals, as these responses include teachers from **four** different class levels from junior high to first year of bachelor's degree, indicating modularity and adaptability to different groups, ages and contexts. Additionally the difficulty was rated easy to medium by the teachers, similarly to the scores given by the students (**Figs 4.2.10, .11 and .12**) and the level of the workshop was rated as very high. More detailed open ended answers are given in **table 4.2.4**, regarding what knowledge and skills were acquired, as well as what was particularly good, bad and could be improved, in both the biology and electronics parts of the workshops.

Question	Biology	Electronics
In your opinion, what knowledge do you think that students have acquired in this part?	Concept of promoter and primer Discovery of techniques and technologies have not been addressed in their program (PCR, transgenesis, etc.). Nature of GMOs The notions of priming and amplification, knowledge about GMOs, **the importance of controls in an experiment** Revision of DNA replication and protein translation Technique (PCR,), simple handling and good laboratory practices (gloves, pipettes...), some notions about GMOs.	no special knowledge Physics knowledge (college technology) I don't know How to solder - **Gaining confidence in the possibility of self-fulfilment, discovery of the existence of fablabs** Notions of fluorescence
In your opinion, what were the skills acquired by students at the end of this part?	They acquired autonomy in carrying out a protocol, which is out of the ordinary. **Autonomy, technicality, analysis, critical thinking, mobilization of knowledge and specific vocabulary, communication, argumentation, etc.** **Scientific method, the use of different controls.** Working according to a protocol; handling with care. Improvement of their pipetting technique, teamwork (working in pairs), compliance with a protocol Handling of small quantities of liquid under clean conditions. Simple handling and good laboratory practices (gloves, pipettes...), interpretation of PCR results.	**Ability to use new equipment according to instructions** Technicality, autonomy, mobilization of knowledge in physics (college technology). Technical skills for the realization of the circuit How to solder **Ability to solder, meticulous work applied with high concentration**
What did you particularly like in this part?	The direct application of theory in practice **The workshop as a whole was perfect in terms of theory, practice, the exchanges, the debate, etc.** **Scientific approach/method, different controls used** **The very visual reading of the results** The slide show and how the GMOs and the handling were presented to the students. I was a little scared at one point during the presentation, given the difficulty of understanding the controls in this manipulation, but as it was really well done, it was easy. The students were really able to follow and understand the necessity of all the controls that were done. (Junior high) Building on the students prior knowledge. The possibility of using this knowledge outside of university (private sphere)	To see students in a new situation **Everything was perfect as a whole!** **The students were happy to actually make the detector** **Cooperation between students in a group** Students' concentration on the task at hand **Simple and quick interpretation of the results (fluorescence reading)**
Were there	no	**Too many instructions at once**

any aspects that you didn't like about that part?	no no The wait time during amplification no no no	**and some components too complicated to weld** None! **I would have liked the students to understand a little bit more about the circuit they have assembled.**
What could be improved in this part	**When giving the instructions include some theory appropriately to accentuate the link between theory and application.** If possible, have another debate on ethical aspects in order to empower students and future citizens, to empower them as future critical players, etc. This debate could continue in a second time (day) with the speakers from the CRI because the message is received differently than with us as their teachers (despite my experience in a research laboratory). Prepare better the "debate" Perhaps having access to student handouts a few days before the session Work on the pipetting before doing the experiment. Improvement of the theoretical part: give more details on PCR, the explanation of the different samples....(Bachelor)	It's up to you! For more autonomy of the students, possibly a circuit diagram with the location of the parts to be placed. All that would be left to do is to validate or rectify before soldering. With a little technical detail on the components of the circuit and their role in the detector. More explanations/details of the theoretical part

Table 4.2.4 Detailed teachers opinions on the workshops

Other questions included: Do you think this workshop has fueled the curiosity of your students? To which all teachers answered “yes”, “yes a lot” or “enormously”.

Some representative responses to other questions asked (translated from French, emphasis mine):

Q: What impact do you think that this workshop has had on students? A: *"Linking the theory learned in class to the reality of doing research. Increasing the appeal of science. Extremely positive on many points. Emphasis on important aspects that are not always highlighted in the classroom. They experienced interdisciplinarity and a window to the world of today. Motivation, opening their mind and giving confidence. Developing attraction for biological sciences and ecology."*

Q:Do you think this workshop has a real pedagogical added value? For what reason(s)?

A:*"Yes, because it is always pedagogically interesting for our students to be confronted with external scientific actors both from a methodological and practical point of view."*

"Yes, for many reasons. The pupils learn differently and in a different framework and context from the usual ones. They are therefore more receptive. This makes it possible to mobilize the knowledge and skills of several disciplines. **It encourages the decompartmentalization of knowledge-competences (contrary to the compartmentalization of disciplines imposed by the national education system) which is necessary and used in the professional environment. This allows for a global approach necessary for understanding many complex mechanisms."**

"Yes, the realization of a real experiment with the material on DNA and the construction of the detector really interested the students while going out of the usual school framework which allowed some to invest more in the project."

"Yes, it is a different time that allows them to understand and reevaluate from another perspective the concepts previously discussed in class."

"Yes. The students are always extremely grateful for the presence of an outside speaker. I could tell them the same thing, it would not have the same impact. The students consider that I'm paid to teach them, so that's my role, period. But when an outside speaker comes, they really come for them, and that's important to them. They are always more involved with an outside speaker. "

"Yes, the workshop is part of several themes of the 1S programme (genetics and feeding humanity), it allowed revisions on the one hand, and to go beyond the programme on certain points of genetics and to initiate reflection on the use of GMOs in agriculture."

4.2.6 Comparing high school experiments that worked and those that did not.

High School workshops were conducted in a short time period, (see **table 4.2.1**) and close to when we were moving labs. Unfortunately this caused some kind of contamination or destabilization of the reaction, such that there were many false positives as well as negatives, when conducting these experiments (which were not freeze dried, and before robustness optimization (See sections 3.2.2, 2.3.5

respectively)). Half of the reactions worked well (73 out of 145), when allowing up to one mistake in any of the first six control tubes, i.e at least 5 of the 6 controls worked as they should have (see experimental setup **Fig 3.2.6**). This quasi-experiment allows us to compare the reported results of the students where the experiments worked as expected and those where it did not.

Failure has been argued to be one of the most essential parts of actually practicing real science (Firestein, 2015) and is almost never discussed. A related and critically neglected goal of practical school science laboratories is for participants to understand the complexity and ambiguity of empirical work (National Research Council, 2006). Interestingly we find no significant difference between the students where experiments worked well, and those for which it did not in terms of self-reported knowledge, skill mastery, and attitudes when these two subgroups are compared (**Fig 4.2.13, A B** and **C** respectively). These results suggest that students learn (or at least perceive that they learned) just as much from a failed experiment as from a successful one.



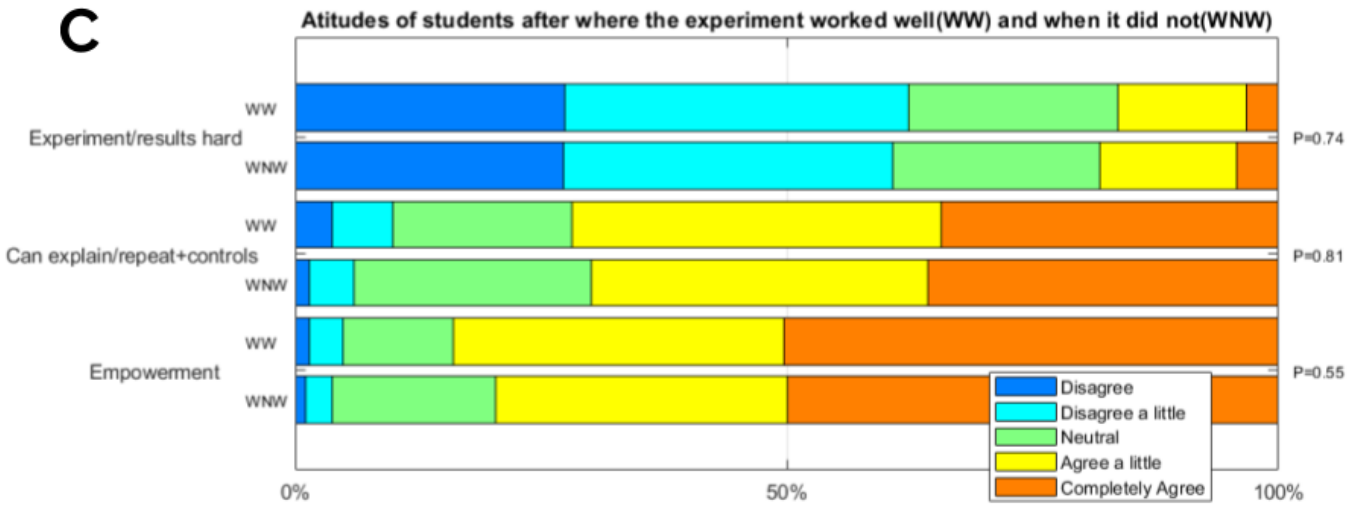


Figure 4.2.14. Students report similar learning and empowerment gains irrespective of a lab experiment working as intended. Student responses(N=145) to pre(**A**) and post(**A, B** & **C**) workshop questionnaires on a 5 point Likert like scale. P-value of a two-sided Wilcoxon rank-sum test between the two groups' answers. Pooled responses of 3-7 questions (items) with Cronbach's alpha >0.8 for all scales. **A) Can you explain to a peer/relative the following notion:** What is a GMO? DNA amplification techniques? Fluorescence? And Citizen Science? IDK corresponds to "I don't know" **B) Did you master the following skills:** Using a micropipette? Conducting a scientific experiment? Working with a group? Formulating ideas? Reflecting on a socio-scientific subject with a critical eye? Generating and analyzing data? Connecting several disciplines/subjects together? Taking part in a citizen science project? **Do you agree or disagree with the following: 1)** I found the experiment hard to achieve' 'I found the experiment hard to understand' 'I found the results hard to interpret' **2)** 'I can explain what's in each of the 8 tubes' 'I can explain what a negative control is' 'I can explain what a positive control is' 'I am able to repeat this experiment with someone else' **3)** 'I feel comfortable with the realization of a scientific experiment' 'I discovered that there are plenty of resources at my disposal to conduct my own actions' 'I know what a Fablab is and what can be done' 'I learned the importance of sharing knowledge''I learned that I could take part in scientific research' 'I was interested in what I did and learned' 'This workshop made me want to do some research'.

4.3 Discussion

My results indicate at the very least that there is a place for this kind of intervention, if only for the enthusiastic responses of so many diverse participants of many ages (12-75) and backgrounds to the assertions: I discovered that there are plenty of resources at my disposal. I learned that I could take part in scientific research. And I was interested in what I did and learned. Additionally it seems all of the goals of a

practical science lab were achieved (National Research Council, 2006), in a challenging and understudied but extremely relevant subject (Tibell & Rundgren, 2010), without the need for any expensive or complex apparatuses. Enhancing mastery of multiple subject matters as attested to by the improvement in self reported ability to explain all relevant concepts. Developing scientific reasoning and understanding the complexity and ambiguity of empirical work and the nature of science as seen by the responses to questions regarding controls in experiments, and through discussions on the results and the need to repeat experiments multiple times in order to be confident of results. Developing practical skills such as using micropipettes, soldering and building your own tools, as well as conducting a scientific experiment and reflecting on a socio-scientific subject with a critical eye. Cultivating interest in science and interest in learning science, especially in subjects they knew little about before and not just passively, but also willing to actively invest time in these subjects. And finally developing teamwork abilities, as could be seen when participants had to hold either tubes or a PCB while teammates tried to pipette or solder small volumes or components carefully and precisely (and without burning anything or anyone or contaminating a wrong tube). In a formal setting teachers were especially satisfied with the practical aspects as well as the appropriateness to the level of the class and the fit to the curriculum, and highlighted the active and interdisciplinary aspects which facilitated "...the decompartmentalization of knowledge-competences, contrary to the compartmentalization of disciplines imposed by the national education system…(which is) necessary for understanding many complex mechanisms."

Despite these successes there is only so much that can be accomplished in a 3-6 hour framework, and this study suffers from some weaknesses. The design is neither experimental or even quasi-experimental, with no control group. Although having another class not receiving the intervention would be unethical (Shawa, 2017) a comparison to a similar PCR based experiment might be insightful. Additionally all participants were already interested in these subjects at some level, as they chose either to come and give their time or to study these topics in school. Conducting similar workshops with other publics such as art, computer, business, history students, or professionals could show different findings. I focused here on self reported knowledge, understanding and skill mastery but these were not checked

explicitly. Questions could be derived which aim to assess if participants really can explain DNA amplification, transgenesis, or the use of controls in experiments, and pipetting precision could also be tested, although these become tedious and were not really what we hoped to transfer to the participants. These are all difficulties commonly encountered in learning research especially in informal settings (Bell et al., 2012; National Research Council, 2006). Retention was also not checked, although some teachers responded weeks after that students' curiosity was raised and they kept asking questions, in addition to multiple participants contacting me after workshops to discuss various aspects. Finally integrating this into a wider interdisciplinary integrated instructional unit (National Research Council, 2006) sequence (Bybee, 2010) or learning progression (Duncan et al., 2009; A. Todd & Kenyon, 2016), as one school did, connecting this with classwork on DNA and genetics concepts, as well as a unit on feeding the world, other units on bioethics, fluorescence and light, electronics, and even english as a second language could prove even more beneficial.

Chapter 5:

Conclusion and perspectives

5.1 Discussion

This multi and interdisciplinary work combining and integrating molecular, digital, physical, educational and scientific tools in novel ways had two main goals 1) Lowering the bar of entry, allowing anybody anywhere to detect a specific piece of DNA and 2) To demystify, empower educate and inspire, multiple different publics, by allowing them to make DNA real, seeing it with their own eye, and to supply tools which allow them to build confidence, skills and agency with their own hands.
The focus on robust isothermal methodologies, dismisses the need for expensive precise machinery, highly trained personnel, and specialised laboratory settings. Takes these tools out of the ivory tower and makes them available and easy to use especially in lower resource settings. These new tools and tricks have been shared openly and in an accessible way, could allow DNA detection in a school, a kitchen, an office, a slum or a field. Allowing anyone an entry point to engage in a meaningful way with the rapidly rising importance of biotechnologies worldwide.
In a world with ever increasing educational gaps, exposing an ever growing population of learners to modern life sciences, by doing, emphasising experimental design, data analysis, critical thinking and fun is essential. I hope that this module and methodology developed during my PhD will be one brick of many (A. Huang et al., 2018; Y. Kafai et al., 2017; Kuldell, 2007; Stark et al., 2018; Staub et al., 2016) to support this crucial objective.

5.2 Perspectives

5.2.1 Other contexts: Intergenerational, museums and libraries

One especially rewarding aspect of this work which I have not yet discussed was seeing this tool used in a familial and intergenerational context multiple times. All informal workshops included at least one pair of parent and child (with the children being any age between 5 to 50). Creating unique and intimate interactions and discussions (**Fig 5.2.1**).



Figure 5.2.1 Three generations of a family interacting with GMO Detective and each other.

This particular image was taken (with permission) during the French Fete de la Science (Science holiday/celebration), where we employed a distinctly different 'rolling' (one in one out) format which could be closer to what might be adopted in some museum type settings. Briefly, families were invited to come and do the experiment and were guided personally by one of the facilitators (this format might

require slightly more personnel depending on the number of participants). Children who saw others smashing up food with the pestles immediately wanted to join in. The ideas behind the experiment were explained to the family as they were collaboratively following the protocol. With this close guidance, groups were able to prepare 8 tubes for incubation in less than 20 minutes and then were invited to return an hour later to check on their results, take a photo and discuss further. The brevity did mean less time was available to discuss more deeply different aspects but seemed very successful in engaging younger children and their families. More generally by eliminating the need for a dedicated lab and using either this model or a more classic workshop models (section 4.2.2), the methodology developed in this thesis allows engaging multigenerational groups in a new and exciting way which could easily be adopted by a variety of social public-facing institutions such as museums, libraries, youth/afterschool programs, and NGOs. This has already begun in Spain with two different groups that have adopted both these models in their public outreach (*BBK Open Science*, n.d.) https://bbkopenscience.com/ and http://cesaretopia.com/wetlab/ (*BBK Open Science*, n.d.) and now also in Zimbabwe (https://noorexponential.com/) with more discussions and interest growing.

5.2.2 Scaling up

In order to do the workshop now people must come to us or we to them, this is not sustainable, especially if we want many people in different countries to do the experiments, learn, and contribute data. We are actively trying to remedy this and further lower the barriers to participation. We can group the participants into three broad groups we are focusing on: High schools, Biohackers/Biocurious (people interested and with access to a lab), and the general public. For all these groups we have planned and have scripted a mini MOOC (Massive open online course) in collaboration with https://syntheticbiology1.com/ and the MOOC factory http://mooc.cri-paris.org/. The basic (core) miniMOOC will consist of short standalone (modular) videos on: How to order hardware and consumables; How to order wetware (enzymes, primers); Building your own hardware; Basics of fluorescence; How QUASR and LAMP work; What we can and cannot detect using this; Overview and explanation of the protocol; How to pipette; A whole video protocol; Analyzing the results; and Intro to citizen science and sharing results.

These short videos will also be useful in other contexts we believe and will be integrated into the synbio1 course as well. As a further elaboration, we are considering adding advanced videos/tutorials for specific audiences such as designing new LAMP primers for biologists. For teachers videos on why this is a good fit for schools; How to integrate it within their program; How to create interdisciplinary content (looking at problems from different perspectives); How to connect this workshop to Biology, Physics, Philosophy, Ethics, Social studies and Civics; Having productive debates in class; and Alternative education structures such as Fablabs. We are concurrently working on improving and making available the documentation for all the different parts, so that everything is clear and can be reproduced simply. We are also considering different ways to make available for direct purchase both the fluorescence detector as well as the freeze-dried reactions.

We are planning on sharing the workshop with as many community labs as we can. I have done three different workshops at the Global Community Biosummit in the MIT Media lab, where community biologists come together once a year. For this, we are also building a training program and documentation to allow new people to learn how to give the workshop themselves. Some labs have already given the workshop themselves or expressed interest in doing so (http://www.hackuarium.ch/en/, https://reagentlab.org/en/, https://sound.bio/) We have also gotten interest from a few different groups that would like to do the syntheticbiology1 course together and this would be one of the practical lab exercises.

5.2.3 Scaling out

Finally I am very excited about expanding this project to ask new questions in other domains in an open participatory and collaborative way. Muki Haklay defines 4 levels of participation and engagement in citizen science projects (Haklay, 2013),
1) ‘Crowdsourcing’ where citizens act as sensors or volunteer resources.
2) ‘Distributed intelligence’ where citizens act as basic interpreters or volunteer thinking.
3) ‘Participatory science’ with participation in problem definition and data collection.
4) ‘Collaborative science’ where problem definition, data collection and analysis is

collaborative and requires that scientists act as facilitators, in addition to their role as experts.

LEVEL 4 - 'DIY Science'	Collaborative science - problem definition, data collection and analysis
LEVEL 3 - 'Participatory science'	Participation in problem definition and data collection
LEVEL 2 'Distributed intelligence'	Citizens as basic interpreters
LEVEL 1: 'Crowdsourcing"	Citizens as sensors or contributing computer resources

This highest level is where I am aiming at taking the project. We are planning on sending out a call to diverse communities all around the world asking them what are their questions, what genetic sequences they would like to look for. We will help the most motivated communities and those with the most interesting questions in developing new primer sets which they will test, benchmark use. Preliminary conversations and interests include

Other food targets:

Other food related targets would be the most straightforward way to extend this work. LAMP primer sets for a variety of relevant targets have already been published such as for the "five forbidden vegetables" onions, garlic, chives, green onions and leeks (M.-S. Lee et al., 2017), which some Bhuddist don't eat. Other dietary restrictions such as pork or horse meat (Ran et al., 2016; Tasrip et al., 2019) and common allergens such as celery (Zahradnik, Martzy, et al., 2014), peanut, sesame or soybean (D. Yuan et al., 2018).

Environmental and ecological targets:

Stemming the tide of global species decline requires continuous monitoring and nimble, adaptive management to promote species recovery (Natesh et al., 2019). A crucial first step for reducing human carnivore conflict and the illegal wildlife trade is identifying the species. This is especially difficult for cryptic/elusive, endangered/rare, sympatric, and morphologically similar species such as tigers (Panthera tigris) and leopards (Panthera pardus). This is currently commonly achieved using molecular techniques such as polymerase chain reaction (PCR) (Maroju et al., 2016) or sequencing (Caragiulo et al., 2015), but would be easier and more accessible if our methodology was adopted. Other endangered invasive or elusive plants or animal species could also be detected such as pangolins (Willcox et al., 2019), carp (Martinez et al., 2020), or sharks.

In addition the detection of harmful microbes (Kundapur & Nema, 2016) could also be achieved. Panels based on local knowledge and needs could be put together to assist farmers and local officials in monitoring plants (Mohammad Amin Almasi et al., 2013; Cassedy et al., 2019; Lau & Botella, 2017; Ocenar et al., 2019; Tomlinson et al., 2010), livestock (Dhama et al., 2014b; Nemoto et al., 2011; Sahoo et al., 2016; Segawa et al., 2014b; Yang et al., 2018) or water (X. Huang et al., 2018; Jiang et al., 2018; Pecson et al., 2020; Williams et al., 2017) for common pathogens and pests, allowing them to respond promptly before a problem arises.

Using the work in this thesis along with the materials we are developing and other open tools which are available, other groups could design their own assays which are especially relevant to them locally or globally. Models could include an advanced undergraduate biotechnology course where students first decide on a target, do the bioinformatic work to identify relevant regions (conserved but distinct) and design primer sets to test, then optimize. Some preliminary discussions such as these have already begun. A local community biology lab developing its own assay such as (*CitizenSalmon*, n.d.) working on tests to differentiate different salmon species. Or a more global collaboration bringing together different actors through new tools designed especially for these kinds of endeavors such as JOGL (Just One Giant Lab (*Just One Giant Lab - Learning and Solving Together*, n.d.)).

Epilogue

Covid 19 update : As a global pandemic has swept throughout the world and suddenly millions of people have learned about PCR based specific nucleic acid detection (and some of its inadequacies) this work has become even more relevant. Through the above mentioned JOGL platform a large team of scientists and citizens from around the world have come together to help create open source and collaborative solutions to issues related to SARS CoV2 (see https://app.jogl.io/program/opencovid19). As part of this effort I have contributed to multiple teams working on the Diagnostics and Detection Challenge (https://app.jogl.io/challenge/opencovid19-detection). Spearheading a project which takes all the aspects of this thesis (as well as adding reverse transcription, other RNA related issues and biosafety into account) to create CoronaDetective (https://app.jogl.io/project/181/CoronaDetective and submitted manuscript below) Additionally I am closely advising a team in Sri Lanka on developing their own diagnostic, aimed mainly at lowering testing costs and deployment in the only international airport in order to reopen the country to tourism.

Our Corona detective is also currently converging with a very similar project (https://app.jogl.io/project/187/COVIDALERT) that has advanced to the finals of the global XPRIZE Rapid Covid Testing (https://www.xprize.org/prizes/covidtesting) and we are all in preliminary stages of clinical validation, offering an open source test which costs less than 2$ and is simple and scalable and bringing me full circle back to where this all began.

Finally with global lockdowns and students confined to their home or to small groups GMOdetective has been piloted as a part of novel at home active laboratory course, on DNA, RNA and Enzymes, as a stand alone module on DNA in Chile (see https://osf.io/ezwhm/wiki/home/ in spanish) .

Corona Detective: RT-LAMP based simple scalable and robust SARS-CoV-2 detection method

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Abstract Surveillance screening to identify people infected by SARS-CoV-2 prior to extensive transmission at scale is key to bringing an end to the Covid-19 pandemic, even though vaccinations have already begun. Here we describe Corona Detective, a sensitive and rapid test based on Loop-mediated isothermal AMPlification to detect the virus, that could be applied anywhere at low cost. Critically, the method uses freeze-dried reagents, readily shipped without cold-chain dependence. It includes a DNA polymerase capable of strand displacement at a single temperature and primer sets to detect the viral N gene and a control cellular RNA, both with sequence-specific quenched-fluorescence readouts. Corona Detective can be used in 8-tube strips to be read with a simple open-design fluorescence detector. Other methods to use and produce Corona Detective locally in a variety of formats are possible, and already openly shared. Detection specificity is ensured through inclusion of positive and negative control reactions to run in parallel with the diagnostic reactions. A simple user protocol, including sample preparation, and a bioinformatics pipeline to ensure viral strains will still be detectable with chosen SARS-CoV-2 primer set, complete the method. Through rapid production and distribution of such reactions, quite inexpensive at scale, daily or weekly surveillance testing of large populations, without waiting for symptoms to develop before testing, is anticipated, in combination with vaccination campaigns, to finally control this pandemic.

Keywords:

Computational Biology

COVID-19

Fluorescence

Molecular Diagnostic Techniques

Open-Source

RT-LAMP

SARS-CoV-2

Introduction

The recent SARS-CoV-2 epidemic has revealed fragility in public health preparedness. The pandemic requires a fair, rapid, large-scale deployment of biological testing beyond what the existing biomedical infrastructure was able to supply early in 2020. Despite enormous efforts across public and private sectors, detection of SARS-CoV-2 in people or in the environment remains cumbersome and expensive(Vandenberg et al., 2020). Currently used techniques of molecular amplification are costly and require complex infrastructure and biosafety procedures, limiting diagnoses of people everywhere. Even with vaccines already being administered, testing remains a crucial factor to control the current pandemic, with prompt surveillance testing of populations particularly important, as 40% of potential carriers are asymptomatic and as new virus variants evolve. Sensitive alternatives to the 'gold standard' for molecular diagnostics of this RNA virus, reverse transcription quantitative polymerase chain reactions (RT-qPCR), with a simpler reaction methodology and reagents that can be readily shipped without cold-chain dependence are necessary.

The Corona Detective described herein is based upon Reverse Transcription Loop-mediated isothermal AMPlification (RT-LAMP). LAMP rivals PCR for sensitivity, without requiring thermocycling or even a cold chain (Carter et al., 2017; Thai et al., 2004). Multiple groups have recently developed RT-LAMP assays for detection of SARS-CoV-2(Augustine et al., 2020), (see review in this journal). Because the majority of these reported methods rely on using non-specific readout methods, such as intercalating dyes or a change of pH, they cannot readily distinguish spurious amplicons that frequently arise during isothermal amplification (Gonçalves et al., 2019; Jiang et al., 2015), increasing the risk and rate of false positives.

This shortcoming calls for the use of sequence-specific detection methods(Becherer et al., 2020), such as those used in in the first RT-LAMP test to receive an Early Use Authorisation from the FDA (Atila BioSystems, Inc., 2020) (iAMP® COVID-19 Detection Kit) or QUASR (Quenching of Unincorporated Amplification Signal Reporters)(Ball et al., 2016; Priye et al., 2017). This method employs a fluorescently-tagged oligonucleotide from the main LAMP set (FIP/BIP or LF/LB) and a short oligonucleotide complementary to the one tagged, but with a quencher the shields the fluorescence when bound, allowing signal to develop from amplicon production. This strategy results in sequence-specific detection and allows multiplexing for the detection of more than one target in a single reaction.
Over recent years, we democratized LAMP-based QUASR detection reactions for use by members of the general public (age 5-75) to learn about and to detect genetically modified organisms in open science workshops (spearheaded in the GMO Detective project (*GMO Detective – an Open, Affordable, Reliable, Easy to Use, Rapid, and Robust Kit for Gene Detection by Non-Scientists*, n.d.). To this end, we optimized speed, sensitivity, and usage robustness (Aidelberg, in preparation), and also designed an affordable ($2) and easy-to-build open-source hardware fluorescence detector(*GMODetective-Detector*, n.d.). This earlier work enabled a quick adaptation of this QUASR method for SARS-CoV-2 detection. As an open science collaboration with Just One Giant Lab (JOGL) and its international team of collaborators, including the CRI (Paris) and the open public lab, Hackuarium (Lausanne), do-it-together participatory research brought together the Corona Detective project (Quero F Aidelberg G Aronoff, n.d.). One key innovation of the method, which uses freeze-dried reaction components, is the ability to provide Corona Detective anywhere readily, with no cold-chain dependence and in a variety of shelf-stable formats.

This means scaling up this method can be truly possible. That the method can be readily adapted to various targets, additionally, means that this work may also provide better pandemic preparedness for the future.

Results

Reaction design and optimization

Research to develop the Corona Detective was openly documented throughout(Quero F Aidelberg G Aronoff, n.d.) as part of the JOGL OpenCOVID19 Initiative. Detailed, openly annotated protocols for making(Aidelberg & Aronoff, 2020) and using (Aronoff & Aidelberg, 2020) the Corona Detective are available. In brief, RT-LAMP primer sets targeting the nucleocapsid (N) gene, the most abundant viral RNA in infected cells (Finkel et al., 2021), and also primers targeting cellular human mRNAs for use as internal controls were initially compared. A plasmid encoding the N gene and a synthetic RNA control for SARS-CoV-2 were obtained (IDT, BEI, respectively) and used to optimize reaction conditions, in particular relative primer ratios and magnesium concentrations, while comparing reactions with one of two polymerases, Bst LF or Bst 2.0 and the RTx reverse transcriptase (NEB). Real-time amplification data was acquired, using SYBR-Safe intercalation for detection, as described (Carter et al., 2017).

Two primer sets, termed NM, for the viral N-gene from the Mammoth protocol with the RNAseP POP 7 set (RP) (Broughton et al., 2020), were chosen for reaction optimization from these early empirical tests. Examples of real time data are presented (Fig. 1), with amplification of the control plasmid N gene (Fig. 1A), and reverse transcription and amplification of the control synthetic RNA (Fig. 1B). The Bst 2.0 enzyme is more rapid than the original Bst LF polymerase, as expected, with faster time to detection threshold (16 vs. 22 min). It also tolerates decreases in magnesium down to 5mM, unlike Bst LF, which would not amplify target under those conditions. Late non-template amplification by Bst 2.0 was evident, however, although this was only rarely observed with Bst LF. Amplification of synthetic target RNA was readily observed, with an apparent limit of detection of about 1 copy per microliter of the Corona Detective reaction or 20 copies (Fig. 1B).

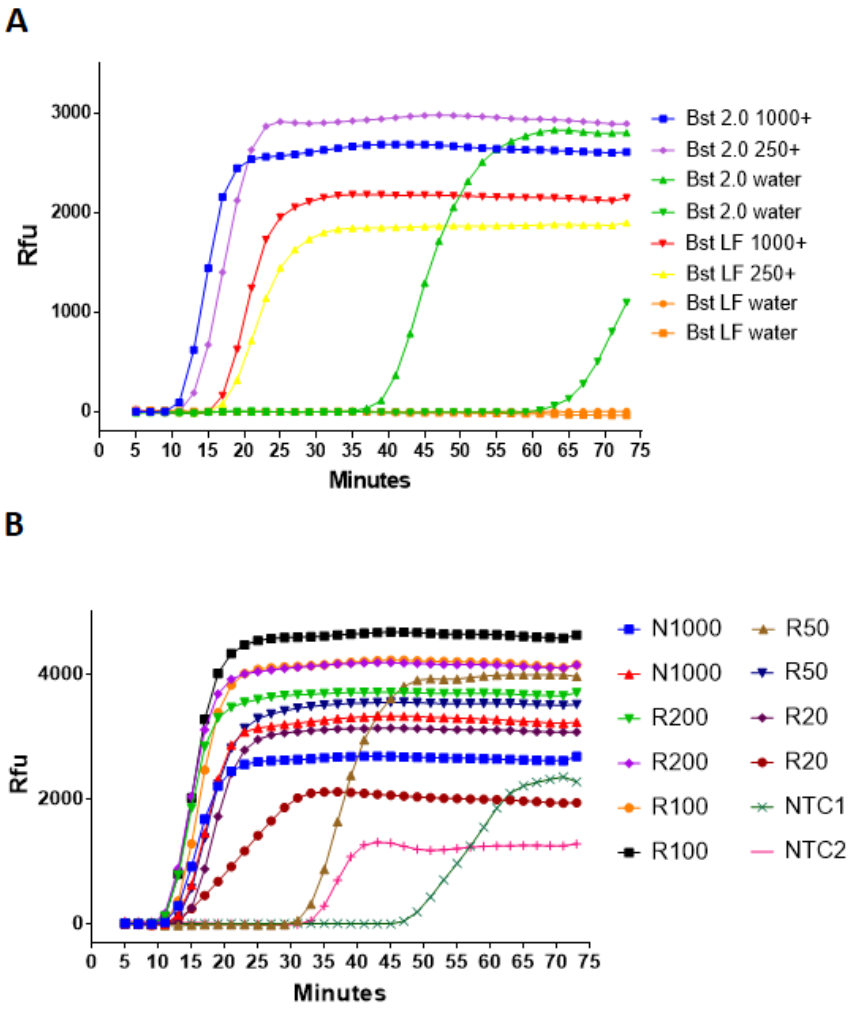

Figure 1: Real time amplification data shows rapid and sensitive detection of SARS-CoV-2 N gene.

A: Bst 2.0 is more rapid than Bst LF, but also more prone to non-template amplification. N250/N1000 reactions have 250/1000 copies of the N plasmid control, respectively. The non-template control (NTC) was water. Incubation temperature was at 64ºC and reaction volumes were 20µl.

B: RNA controls are reverse transcribed and amplified for detection down to a single copy per microliter of the reaction. Synthetic BEI RNA dilutions were spiked into duplicate reactions at 20, 50, 100 and 200 (R20, R50, R100 and R200) copies, in addition to NTC and DNA controls. These reactions used Bst 2.0 and RTx, incubation included an initial 10min@55ºC for reverse transcription before shifting up to 64ºC for the real time acquisition.

Optimized reaction conditions from such experiments used primer concentrations of 1.6, 0.8, 0.2 µM respectively, for FIP/BIP, LF/LB, and F3/B3, and a magnesium concentration of 7mM, while aiming to avoid non-template control (NTC) amplification (Table 1).

MgSO4	**Bst LF (min.)**	**Bst 2. (min).**	Fastest time observed (N1000) in minutes

5mM	na	27	na=no amplification
6mM	35	21	
7mM	31	19	Both NTC up for Bst2.0, but not Bst LF at 7mM
8mM	21	16	both NTC up for Bst LF and Bst2.0 at 8mM

Table 1: Magnesium optimization of Corona Detective.
Concentrations of magnesium sulfate (MgSO4) were varied in reactions with either of the two polymerases (Bst LF or Bst 2.0) The fastest time to threshold in minutes for 1000 copies of the N plasmid (N1000) is reported.

Sequence-specific fluorescence detection

We designed QUASR primers for the NM primer set, termed NM*FAM, adding the FAM fluorescent tag to the 5' end of the FIP primer. A short complementary quencher primer, of 11 bases, with the quencher at its 3' end (sequence 5'CATTGGCCGCA-Q) was also synthesized. When annealed to the tagged FIP primer, this quencher prevents fluorescence, but when the FIP is incorporated in the amplicon, this tag fluoresces freely, providing the basis for strong QUASR detection. The internal human control, RP, was detected via a QUASR design used by others already (Curtis et al., 2018), with a 13mer attached to a quencher, complementary to the 5' end of the RP LF primer, tagged with another fluorophore (HEX).

The QUASR fluorescence cannot be read at amplification temperatures, but requires lower temperatures (<~35°C), to allow the quencher to anneal to unincorporated tagged primers and prevent fluorescence. It is thus used for endpoint detection. With the simple fluorescence detector from the GMO Detective project(*GMO Detective – an Open, Affordable, Reliable, Easy to Use, Rapid, and Robust Kit for Gene Detection by Non-Scientists*, n.d., *GMODetective-Detector*, n.d.), illuminated by blue LEDs and with amber filters in front of the tubes, controls were readily detected after 10 minutes incubation at 55°C and 30-90 min at 64°C (Fig. 2). Furthermore, detection of both targets in the reactions was sensitive and reliable, with a limit of detection of 20 RNA copies per reaction for the NM*FAM reaction (Figure 2A). In this detector, the HEX signal is somewhat more orange than the green FAM, particularly by eye, and the multiplex reactions are indeed visible in the simple detector (Fig. 2B). A standard phone camera is sufficient to capture images, another bonus of the QUASR method (Fig. 2).

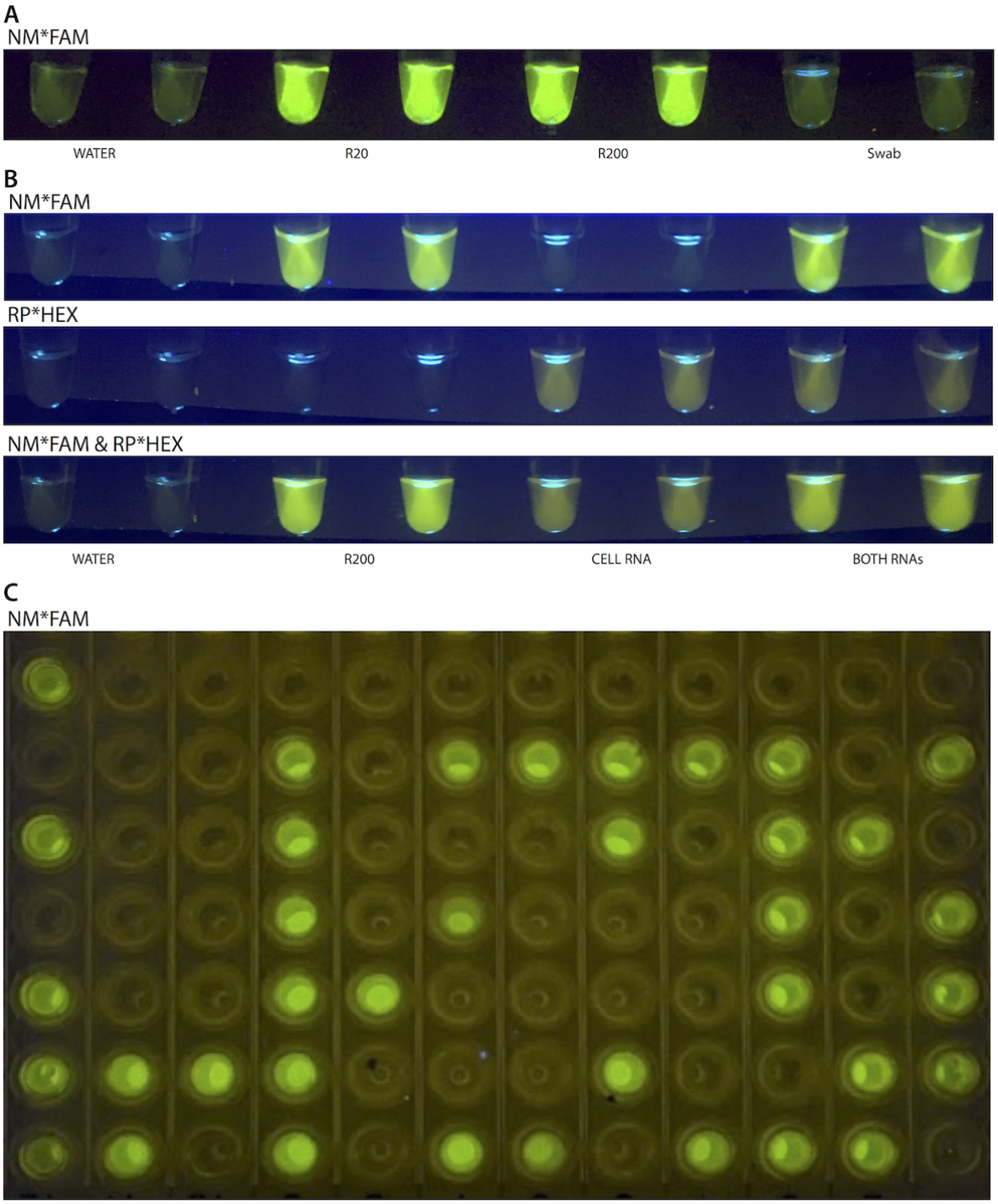

Figure 2: QUASR Detection of Corona Detective

A: NM*FAM gives positive results down to 20 copies of the BEI synthetic RNA (R20) per reaction. No positives have yet been observed from no template controls (water) or from environmental swabs of doorknobs.

B: The multiplex reactions (NM*FAM & RP*HEX, bottom) sum the single reactions, above, and are distinguishable even in this simple fluorescence detector.

C: A 96-well plate imaged on an affordable blue light gel box transilluminator, one month after reactions were run. Images acquired with standard smartphones.

The most encouraging finding from these multiplex QUASR results was that the signals came up well with high sensitivity, but without problems of false positives, even though the Bst 2.0 enzyme was used for these reactions, rather than the slower Bst LF, and even with incubation of up to 2 hours. Overall, complete elimination of false positives in over 1000 reactions lends great confidence in this QUASR detection, furthermore, results are stable for at least 1 month when stored in a cool dark place (Fig. 2C). An additional strength of our detection method includes affordability and ease of detecting the sequence specific amplification. As mentioned this can be done using an open source(Andre Maia Chagas et al., 2020) DIY fluorescence detector for 8-strip tubes(*GMODetective-Detector*, n.d.) (Fig. 2A and B). For whole 96-well plates off-the-shelf blue light transilluminators affordably and easily can detect the fluorescent signal (e.g. https://iorodeo.com/ or https://www.minipcr.com/) (Fig. 2C), or if available, a standard gel imaging system or qPCR machines can also be used, all at room temperature.

Sample preparation and reaction specificity and sensitivity

For rapid, affordable and simple screening especially in lower resource settings, we decided to focus on so-called “direct” saliva sample processing methods (Bastos et al., 2021). The TCEP method (Rabe & Cepko, 2020) for rapidly inactivating and opening virions and reducing viscosity of saliva sample worked best. Benefits of the TCEP strategy include the low volume needed, which prevents sample dilution (1:100), use of only one additional temperature (5 minutes at 95℃), and the buffer’s room temperature stability. A single copy of the synthetic BEI RNA per microliter of reaction was readily detected by such ‘direct’ Corona Detective assays. The limit of detection for this assay was also determined with viral surrogate particles (Zeptometrix) in various matrices (saliva, PBS) and with actual inactivated viral particles (BEI Resources). A panel of other viral RNA controls (Twist synthetic RNAs, for e.g. measles, mumps, influenza and other coronaviruses) were tested and no cross-reactivity was observed, in concordance with the EUA report(*EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE COLOR SARS-COV-2 RT-LAMP DIAGNOSTIC ASSAY*, n.d.) for the NM primer set. Limits of detection of 1-2 copies of SARS-CoV-2 per microliter of reaction were consistently observed (currently 4µl input in a 20µl final volume).
Improved sensitivity may be achieved by using larger input volumes of treated sample per reaction, or pre-concentration of viral RNA from samples, using commercial kits (*CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel - Instructions for Use*, n.d.) or magnetic beads (Kellner et al., 2020; Oberacker et al., 2019). An affordable glass milk method (Rabe & Cepko, 2020) did not scale well to microplate format in our hands. To note: sample collection has its own set of issues, and additional biosafety concerns exist until the samples are at least inactivated.

Freeze drying and manufacture

To reduce costs associated with transport, storage and manipulations, and to allow use even where no cold chain is available, reactions were lyophilized. Briefly glycerol-free enzymes, along with dNTPs, primer mixes, and trehalose as protein stabilizer (Colaço et al., 1992) were added and freeze dried (Aidelberg & Aronoff, 2020; Carter et al., 2017). The

rehydration buffer containing isothermal amplification buffer, MgSO4 and water is also stable at room temperature (for more detail see (Aidelberg & Aronoff, 2020)) These reactions were found to retain sensitivity and specificity for at least 2 months when stored in a cool dark location sealed with a desiccant. Freeze-dried batches have already been utilised after shipping to Sri Lanka (and Chile?) at ambient temperatures, working extremely well after waiting a month on the shelf, for initial clinical validations and further tests.

Bioinformatics

In order to make sure prevalent strains of SARS-CoV-2 are still detectable by the NM primer set, a bioinformatic pipeline is being developed by the team. A 3-step protocol checks for mutations, which might affect recognition by diagnostic primer sets. We conducted analyses, comparing geographical regions, to determine whether any prevalent viral variant may present problems for Corona Detective reactions.

The workflow is the following:

1. Complete high coverage genomes are collected from GISAID EpiCoV™ Database (https://www.gisaid.org/) (Elbe & Buckland-Merrett, 2017).
2. Regional multi-fasta files are aligned using MAFFT online service (Katoh et al., 2019; *MAFFT - a Multiple Sequence Alignment Program*, n.d.).
3. The resulting alignment files in CLUSTAL format are analysed via python functions. (See the GitHub repository of the project) https://github.com/CoronaDetective.

For the current analysis, we downloaded the resulting multi-fasta files categorized by continent (Africa, Asia, Europe, North America, Oceania and South America). Results show the NM primer set target sequences are mutated in less than 10% of sequenced isolates, with the exceptions of the Asian and South American SARS-CoV-2 subsets, where a single nucleotide has been found mutated in two different NM primers. For the Asian sequence a G is mutated to T in the FIP primer target in more than 16% of the isolates, while for the South American sequence a T is mutated to C in the LF primer target in over 30% of isolates (**Figure 3**).

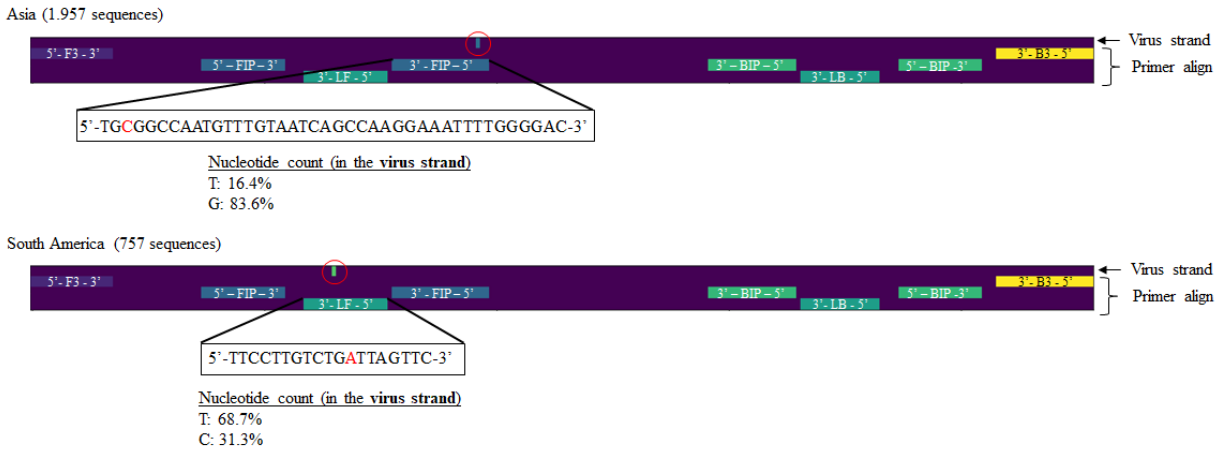

Figure 3: Geographic mutational analysis of sequence variation reveals rare changes in two NM primer targets.
Current analyses from the collected GISAID sequences identified mutations in two different targeted regions of Asian and South American sequenced variants. The rest of the geographic regions analyzed (Africa with 1227 sequences, Europe with 10000 sequences, North America with 10000 sequences and Oceania with 1261 sequences) did not present any relevant mutations (over 10% frequency) for the Corona Detective primers. Analysis was performed upon the complete high coverage sequences submitted between 1 September 2020 and 1 January 2021.

Potential problems of amplification of such new strains, means vigilance to follow their evolution with automatic algorithms, as we describe here, is highly recommended. To note: potential bias in sequencing clusters, globally, means this is certainly not a definitive measure of all the virus evolving currently. Primer sets may need to be changed, eventually to ones which target more preserved genomic regions. Alternatively, two methods can be considered: 1) degenerate or 'wobble base' nucleotides could be substituted into problematic positions of useful primer sequences, allowing them to still interact with the target or 2) the addition of a small amount of a high fidelity polymerase should allow Corona Detective to be less sensitive to mismatches, as has been described (Lu et al., 2020). Additional methods could be used, if, on the contrary, being able to distinguish different strains is desirable (Gill & Hadian Amree, 2020).

Discussion

Done without complex equipment and with a simple fluorescence readout, Corona Detective is an easy-to-use, sensitive, robust and highly specific SARS-CoV-2 detection method. The RT-LAMP primers chosen for the multiplex detection (including the cellular mRNA target as an extraction control) have already been approved for use in two EUAs from the FDA (*EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE COLOR SARS-COV-2 RT-LAMP DIAGNOSTIC ASSAY*, n.d., *EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-COV-2 RNA DETECTR ASSAY*, n.d.) based on RT-LAMP, but with different detection strategies (Crispr-fluor and pH-colorimetric, respectively). Drawbacks of these methods, for instance risks of cross-contamination and sensitivity to buffer effects, are avoided with the robust quenched fluorescence -based detection of the QUASR method. Even if the reaction is left to incubate longer than 45 minutes, false positives in non-template controls are avoided. This is crucial for testing in resource-strapped or point-of-care settings.

Controlling for primer reliability through the described pipeline is so far only done *in silico*, but should help ensure robust detection worldwide. More batches of tubes and 96-well plates will be sent soon to Switzerland, Cameroon, Ghana, Chile, Sri Lanka and potentially more collaborators, made at the CRI in Paris for clinical validation in a variety of contexts. Additional possibilities for large-scale production are already being explored, also in the United States.

To further improveCorona Detective, work has already begun, which aims to utilize in-house produced, enhanced (Maranhao et al., 2020) and/or off patent enzymes (both polymerases and reverse transcriptases (Alekseenko et al., 2020; Kellner et al., 2020; *Open Enzyme Collection – Open Bioeconomy Lab*, n.d.)). Additionally, *in silico* optimization studies are in

progress by our open science team, to increase heat stability and salt tolerance of the polymerase. A point mutation in the tight-binding pocket, anticipated to be more tolerant to modified nucleotides and particularly important for cross-contamination prevention through dUTP incorporation and UTP-glycosidase treatment, will also be examined *in vitro*.

Bottlenecks to rapid scale-up of testing include administrative delays relating to intellectual property and the conservative production infrastructure designed to efficiently supply market needs with limited capacity. These limitations could in principle be overcome through a testing supply chain based upon open-source principles in a distributed, decentralized and locally based way(Gomez-Marquez & Hamad-Schifferli, 2019). The tools for high volume Corona Detective production(Aidelberg & Aronoff, 2020) cost less than an average qPCR machine and could even incorporate locally produced enzymes (Alekseenko et al., 2020; Kellner et al., 2020; *Open Enzyme Collection – Open Bioeconomy Lab*, n.d.) or nucleotides(Loan et al., 2019). Adopting the open Corona Detective in its various formats (8-well, 96-well, 384-well) could help scale up SARS CoV-2 surveillance, particularly in low resource settings, sooner rather than later.

Chapter 6:

Materials and methods

LAMP assays

LAMP assays were initially done using WarmStart LAMP 2X Master Mix and Dye, (#E1700 New England Biolabs) at a total volume of 10µl unless stated otherwise. Later the individual components were separately mixed in order to optimize reactions and for affordability. For a 10µl reaction this includes 1µl 10x Isothermal Amplification Buffer (1X Buffer Components: 20 mM Tris-HCl, 10 mM (NH4)2SO4, 50 mM KCL, 2 mM MgSO4, 0.1% Tween® 20, pH 8.8@25°C. #B0537 New England Biolabs), 3.2 Units of Bst 2.0 WarmStart® DNA Polymerase (#M0538 New England Biolabs), 1.4µl 10 mM dNTP Mix (#N0447 New England Biolabs), 0.6µl 100 mM MgSO4 (#B1003S New England Biolabs) and molecular biology grade water. Primer concentrations were 1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.8 µM LoopF/LoopB. Except in the multiplexing experiment where these were the total concentration of primers but half for one target and the other half for the other target. 0.2µl of NEB Fluorescent dye (Comes with E1700 kit) or SYTO™ 82 Orange Fluorescent Nucleic Acid Stain (50µM diluted in DMSO (1-2µM final concentration) Invitrogen, USA) was also supplemented in most non-QUASR experiments. 2µl of samples/controls were also added. Reactions were usually run for ~60 mins at a constant ~63℃ in a PCR thermocycler unless otherwise stated.

Corn soybean and Food materials.

10%, 1% or 0% Official European Reference Material of transgenic lines of maize (GMO Maize 1507, ERM-BF418D/C/A) and soybean (GTS 40-3-2,

ERM-BF410D/C/A) were purchased from the Joint Research Centre of the European Commision certified reference material (CRM) catalogue. For other percentages of GMO content these were hand mixed by weighing out appropriate amounts. Dried food products containing at least 30 corn or corn flour were purchased from local retailers. These included Tortilla chips nature (Poco Loco, Belgium), 3D's Bugles® Goût Nature (Benenuts/Pepsico France), Reese's Puffs breakfast cereal (General Mills, USA), Frosties® (also known as frosted flakes, Kellogg's, France), and Doritos tortilla Chips from both continents (PepsiCo France and Frito-Lay/PepsiCo North America).

Primers

Primers used in this study were taken from the following references and adapted for QUASR detection according to the instructions in (Ball et al., 2016; Priye et al., 2017).

Primer	Sequence	Target	Citation
FIP	CATCATGCTCGGCAAGACCAGCCTAACATGATGTCACCAT	Maize 10kDa zein	(Bektaş & Chapela, 2016)
BIP	TGCCACAATGTCACTGCGAGGTTGAACATGAATGGTAACTG		
F3	ATGATGCCACAGATGATGAC		
B3	CTATCTAGAATGCAGCACCAA		
FL	TGGTGACATCATGCTCGG		
BL	CCGTCTCGCAGATTATGCT		
BIP-Q	CATTGTGGCA-Q		Here
FIP	AGATGGGTTTTTATGATTAGATTTATCCTAGTTTGCGC	T-NOS	
BIP	TAATTCAACAGAAATTATATGAAGTTTCTTAAGATTGAATCCTG		
F3	CATAGATGACACCGCG		
B3	GATCGTTCAAACATTTGG		
FL	AGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATA		
BL	ATAATCATCGCAAGACCGGCAA		
FIP	CATTGCTTTGCTTCAGCTAAATTGCTGCTAGTATAAATAGGGGCATG	Soy Lectin	
BIP	AACCCAGAATGTGGTTGTATCTCTCAGTTTCCGCTGAGTTTGC		

F3	ACAACTTTTGAAAAGTACCCAAT		(M. Zhang et al., 2013)
B3	GAACTTGTTCCAGCTGAAAG		
FL	ACTGTGATGCATGGGGAG		
BL	CCCTAACCTTAACCTTGGTACTGGTGCTACTGACCAGCAAG		
FL-Q	GCATCACAGT-Q		Here
F3	AAGATGCCTCTGCCGACA	35S	(Zahradnik, Kolm, et al., 2014)
B3	CAGCGTGTCCTCTCCAAAT		
FIP	ACGTGGTTGGAACGTCTTCTTCCCAAAGATGGACCCCCA		
BIP	ATCTCCACTGACGTAAGGGATGATAGAGGAAGGGTCTTGCGA		
FL	TCCACGATGCTCCTCGTG		
BL	ACGCACAATCCCACTATCCT		
BIPQ	CAGTGGAGAT-Q		Here

F3	TATGGGAGCCGTTTTTGC	Cox	(Tomlinson et al., 2010)
B3	AACTGCTAAGRGCATTCC		
FIP	ATGGATTTGRCCTAAAGTTTCAGGGCAGGATTTCACTATTGGGT		
BIP	TGCATTTCTTAGGGCTTTCGGATCCRGCGTAAGCATCTG		
FL	ATGTCCGACCAAAGATTTTACC		
BL	GTATGCCACGTCGCATTCC		
FIPQ	YCAAATCCAT-Q		Here

All Primers were ordered and synthesized by Integrated DNA Technologies (IDT USA/EU) at various scales, but all only with standard desalting, except for short quenching probes (designed here) which need to be HPLC purified. For QUASR detection the corresponding primer to the Q Primer was ordered with FAM (fluorescein) (or CY3/HEX) at the 5’ end, and the Quencher probe with Iowa Black® FQ Dark Quencher at the 3’ end. Primers were kept as either 50 or 100mM stock solutions in water and mixed together at appropriate ratios to create either 10x or 3x Primer mixes.

DNA extraction

When nanograms of DNA are stated, DNA was extracted using the DNeasy Mericon Food Kit (#69514 Qiagen) according to the manufacturer's instructions. DNA was quantified by measuring the sample absorbance on a NanoDrop spectrophotometer. 1-2 µl of each DNA sample was analysed to check the quality and quantity of DNA. This extracted DNA was diluted to different concentrations in water or TE buffer, and kept at -20℃ until analysis. If only volume or sample type is stated, DNA was "extracted" by first smashing and grinding ~5mg of sample using an appropriate polypropylene pestle in a 1.5ml microcentrifuge tube, after which 60-100ul of DNAse free water was added, and the sample was rigorously mixed. This was placed into boiling water for 5 minutes, and 2µl of this was taken as a sample into the LAMP reactions.

Tube Imaging

Tubes were imaged using a gel reader (ChemiDoc™ MP Bio-Rad California, USA) with exposure for 0.1 seconds utilizing the Blue LED illumination (460−490 nm excitation with a 532/28 emission filter) for FAM and Green NEB dye. For Cy3/Hex and SYTO 82 the Green channel was chosen (520−545 nm excitation with 602/50 emission filter). QUASR reactions were imaged when tubes were at room temperature or colder, whereas reactions utilizing an intercalating dye were imaged rapidly when tubes were still warm, as this was found to give a stronger signal.

High throughput/Screening experiments

Custom Matlab code was written which took a standard Definitive Screening Design (DSD (Albrecht, n.d.) and Appendix A), and for each run (i.e. well/experiment) exported the appropriate lines in an CSV file readable by Echo cherrypick software such that the appropriate amounts of each reagent were transferred to the appropriate well, with water added to a total reaction volume of 3µl in a 384 well qPCR plate using an Echo 550 acoustic liquid handler (Labcyte, California USA). Preliminary experiments were done using 3,6, and 9µl of the same reaction to assess if miniaturization of the reactions affected the timing or signal generated in the reactions. No differences were detected. Additionally using the echo a checkerboard pattern of the identical reagents was transferred to half the wells, to assess if any problems of evaporation or edge effects could affect the results, no differences were seen.

Real-time qPCR machine and settings

For high throughput and robustness experiments, LAMP reactions were done in 384 well plates qPCR plates and thoroughly sealed with an adhesive qPCR Plate Sealing film (#38820 and #36590 respectively, Sorenson BioScience, Utah USA) plates were transferred on ice to another laboratory, where they were briefly spundown and placed in a Roche LightCyler 480 qPCR machine. Reactions were run at 63 °C for 90 minutes followed by heating to 95°C for melting curve analysis and then cooling to room temperature for QUASR detection, with fluorescence measurements every minute monitoring both channels (FAM and HEX (here SYTO 82)). Raw data was exported from LightCycler 480 software and a threshold time to positive was extracted using custom Matlab scripts. The median of the four replicates was taken for subsequent analysis. For DSD experiments, statistical analysis was done in JMP® 14 (SAS Institute Inc., Cary, NC, USA) according to (Cary, NC: SAS Institute Inc., 2018; Jones & Nachtsheim, 2017) taking only the significant factors into the model.

Freeze-drying (lyophilization)

Lamp reactions were set up as above, except magnesium and the isothermal amplification buffer were left out. Additionally D-(+)-trehalose dihydrate (#T5251 Sigma Aldrich) was added to help stabilize the components and the enzyme was glycerol free and stored at -80C. For one plate (12 experiment, 96 wells) this included for each primer set: 77ul of dNTPs 10mM each, 55 µl of each 10x Primer Mix ,1.5 µl Glycerol free Bst 2.0 WarmStart® DNA Polymerase 120000 U/ml , 110 µl Trehalose 50 Mass Percent (for a 50% trehalose solution, mix 500mg trehalose with 1mL H20) and 196.5 µl DNAse free water. 8µl of appropriate mastermix was placed in each well, at this point Positive control DNA can also be added. Plates were sealed with a pierceable sealing foil and tops were punctured using a 96-Pin replicator. This is so when the reactions are freeze-dried the small pellets do not “jump” out of the tubes. Plates were then freeze dried using a scientific lyophilizer (Harvest right , Utah USA) which was set to first freeze sample to -40℃ for a few hours then vacuum was turned on aiming for 500mtorr for a few more hours, then sample were slowly heated to room temperature with the vacuum still on. Plates

were then resealed and stored in a cool dark place in a sealed plastic bag with a dessicant.

Open hardware detector design and fabrication

To design our open source detector, we first used Grasshopper plugin for Rhino 3D (Robert McNeel and associates) to programmatically design the 3D shape of the detector, this was then made into different 2D pieces in Illustrator (Adobe). Designed acrylic or wood components were laser cut to desired specifications. Adjustments must be made depending on the laser cutter and thickness of the material to the exact size of the holes/connectors, to make sure they fit snugly together, so the detector is not loose. A variety of different cardboards were also tried and can all work but stability is largely affected by the thickness and weight of the material.

The printed circuit board (PCB) was designed using Eagle software (Autodesk) and ordered from pcbway.com (China), multiple iterations were tested to improve connection to a 5V power source. It was found that a 2 pin MicroUSB connector is easiest to solder (such as: https://www.aliexpress.com/item/1005001515820458.html) and more robust than pins/wires or a standard (data) micro USB 5 pin connector. Although any 5V source should work. Additionally some SMD versions were also tested, although these are much more difficult to solder by hand, they can greatly reduce manufacturing costs.

Affordable optics

A large variety of LEDs and filters were tested. It was found that all blue LEDs with a dominant Wavelength at 470nm, and which were relatively strong (luminous intensity<3000mcd) all gave similar results. The most affordable and easily available LED was chosen (C503B-BAN-CZ0A0451 or 2 Cree®, Inc. Durham, NC USA). An excitation filter is necessary for FAM detection by eye/camera, as the wavelength emitted by the LEDs is not extremely narrow. 2(or 3) orange emission filters are also needed for a clear signal to be detected. Alternatively detection of red fluorescence could easily be detected with no excitation filter using these same blue LEDs, and only 2 red filters (Lee gel filters tested include : 141,716,139, 768,105, 767, 015, 179, 780, 787, 781 and 164). It was found that for FAM detection 716 Mikkel Blue (http://www.leefilters.com/lighting/colour-details.html#716) is optimal as an excitation

filter, and at least two 179 Chrome Orange (http://www.leefilters.com/lighting/colour-details.html#179) are optimal as an emission filter, although 768, 015 and 105 are all reasonable alternatives.
For mRFP1/Tex-Red detection two 781 Terry Red filters (http://www.leefilters.com/lighting/colour-details.html#781) were optimal, with no excitation filter, using the same blue LEDs (Magenta 113 is a reasonable alternative). Filters were cut from larger sheets into small strips, (2x1cm for the emission filter and 1x11cm for the excitation filter) either by hand or also using a laser cutter.

Isothermal Heating

Stability and precision of heating temperature in both PCR machines (Thermocyclers) and water baths (Including Scientific, Sous-vide, and wax heaters) was measured using a Wireless dual channel thermocouple data logger/thermometer (#WW806 Omega, CT USA). Most experiments were conducted either on a T100 Thermal Cycler (Bio-Rad California, USA, or similar), or when out of the lab using a Sous-vide precision cooker (BioloMix Vacuum Sous Vide Cooker 1500W, China).

Workshops

Workshops were conducted as described in the text. High School workshops were led in French by Tonino Rizzio, and facilitated by Guy Aidelberg and Imane Baize. Most other Workshops were led solely by Guy Aidelberg, sometimes with an assistant present, some were conducted remotely such that knowledge of the workshops is limited. Before starting and just after saying hello, pre workshop questionnaires were handed out physically, or a link to a digital version was transmitted. Students received a number and told to remember it for the whole duration in order to anonymize the data, while still being able to compare pre and post questionnaires of the same student. Participants were only encouraged to answer honestly as data was anonymized.
Post workshop questionnaires were similarly handed out, after the workshop was over, a “Prize” in the form of a GMOdetective sticker was given to whoever completed the questionnaire.
Teachers were sent questionnaires electronically ~ two weeks after the workshops were conducted and all answered rapidly. Answers to open questions were

translated to english using a mixed method, combining machine translation (DeepL, Cologne, Germany) followed by multiple native speaker input.

Data Analysis

Data from physical and digital questionnaires was transferred to custom Matlab software for analysis. For sets of questions which were asked both pre and post workshop, data was compared using the Wilcoxon signed rank test for two populations when the observations are paired. Observations for which only one answer was available are omitted for the purpose of P-Value calculation. For questions which were asked only in the post questionnaire, the statistical significance for deviation from neutral of the same Wilcoxon signed rank test is stated. For comparisons between groups of students the Wilcoxon rank sum test was used which assumes that the two samples are independent, and tests the null hypothesis that data in the two samples are from continuous distributions with equal medians, against the alternative that they are not.

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Zhang, W., Xing, F., Selvaraj, J. N., & Liu, Y. (2014). Comparison of DNA Extraction Methods for GM Rice and GM Rice-Derived Food Products In China. *Journal of Food Biochemistry*, *38*(4), 388–396. https://doi.org/10.1111/jfbc.12065

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Zoellick, B., Nelson, S. J., & Schauffler, M. (2012). Participatory science and education: bringing both views into focus. *Frontiers in Ecology and the Environment*, *10*(6), 310–313. https://doi.org/10.1890/110277

Zou, Y., Mason, M. G., Wang, Y., Wee, E., Turni, C., Blackall, P. J., Trau, M., & Botella, J. R. (2017). Nucleic acid purification from plants, animals and microbes in under 30 seconds. *PLoS Biology*, *15*(11), e2003916. https://doi.org/10.1371/journal.pbio.2003916

Appendices

A. HT DSD results

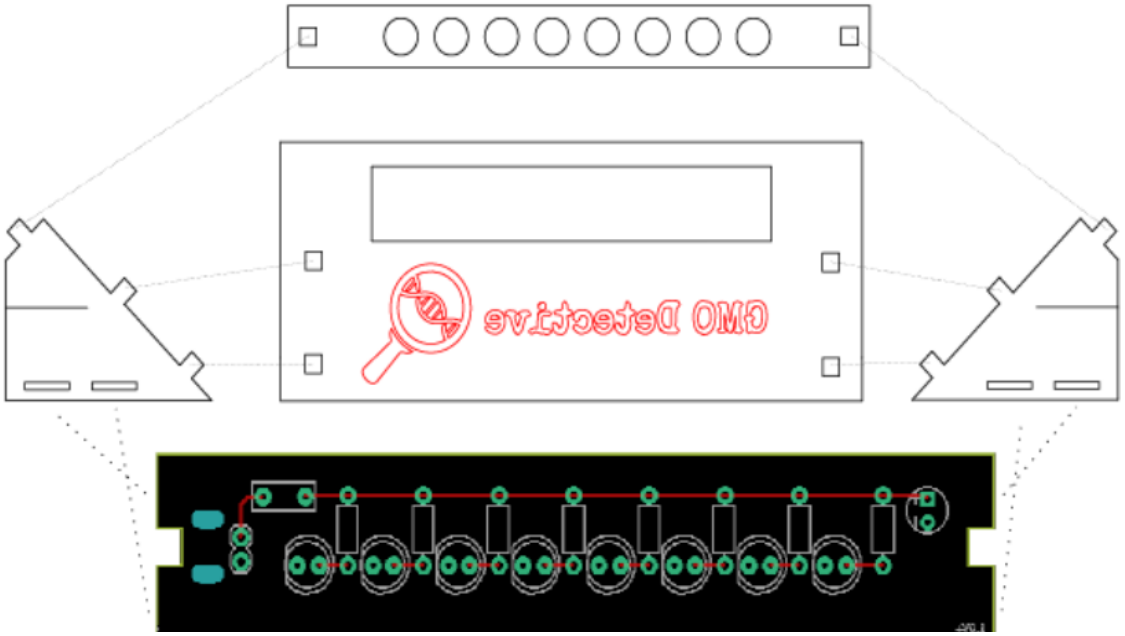
	High(+1)	Medium(0)	Low(-1)
Buffer (X)	1.5	1	0.5
Bst Enzyme (U/µL)	0.32	0.16	0.8
MgSO4 (mM)	10	8	6
dNTPs (mL)	1.4	0.9	0.4
Betaine (M)	0.9	0.45	0
Primer FIP(mM)	1.6	0.8	0.4
Primer BIP(mM)	1.6	0.8	0.4
Primer F3(mM)	0.2	0.1	0
Primer B3(mM)	0.2	0.1	0
Primer FLP(mM)	0.8	0.4	0
Primer BLP(mM)	0.8	0.4	0
Sample+(ng/ul)	5 Soy	5 Corn	2 Soy
Sample-(ng/ul)	5 Fly DNA	0 ddH2O	0 Sink H20

Buffer	Enzyme	Mg	dNTPs	beta	fip	bip	f3	b3	flp 2	blp	sample	Time+
0	1	1	1	1	1	1	1	1	1	1	1	18.2
0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	120.9
1	0	-1	-1	1	-1	1	1	1	-1	-1	1	118.6
-1	0	1	1	-1	1	-1	-1	-1	1	1	-1	33.0
1	-1	0	-1	-1	1	-1	1	1	1	-1	-1	123.2
-1	1	0	1	1	-1	1	-1	-1	-1	1	1	80.7
1	-1	-1	0	-1	-1	1	-1	1	1	1	-1	35.1
-1	1	1	0	1	1	-1	1	-1	-1	-1	1	120.4
1	1	-1	-1	0	-1	-1	1	-1	1	1	1	118.6
-1	-1	1	1	0	1	1	-1	1	-1	-1	-1	50.0
1	-1	1	-1	-1	0	-1	-1	1	-1	1	1	121.3
-1	1	-1	1	1	0	1	1	-1	1	-1	-1	118.6

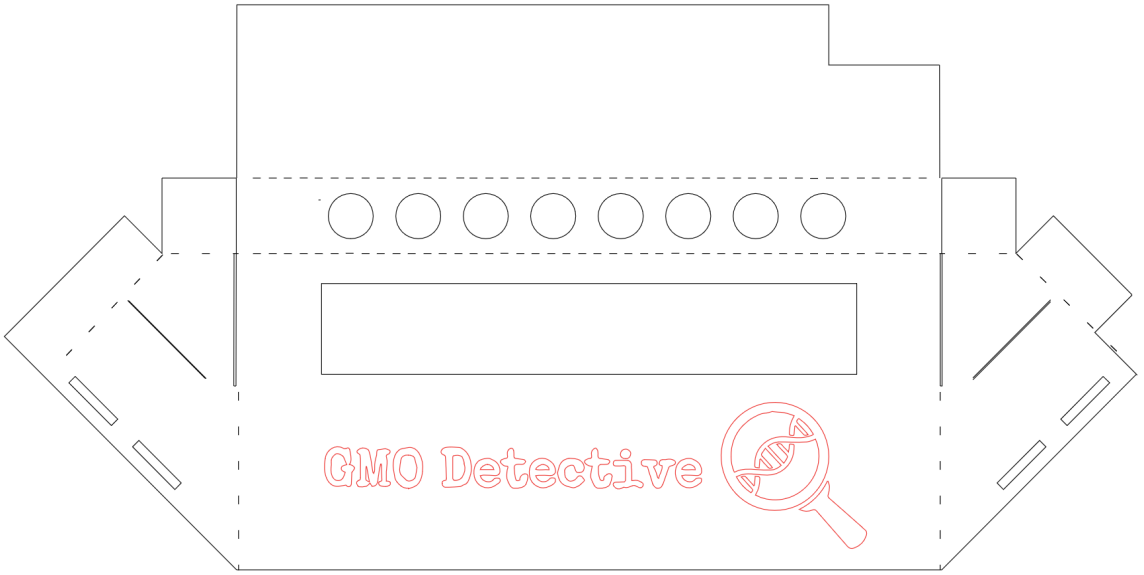
1	1	-1	1	-1	-1	0	-1	-1	1	-1	1	40.4
-1	-1	1	-1	1	1	0	1	1	-1	1	-1	120.1
1	1	1	-1	1	-1	-1	0	-1	-1	1	-1	56.3
-1	-1	-1	1	-1	1	1	0	1	1	-1	1	119.2
1	1	1	1	-1	1	-1	-1	0	-1	-1	1	120.4
-1	-1	-1	-1	1	-1	1	1	0	1	1	-1	37.3
1	-1	1	1	1	-1	1	-1	-1	0	-1	-1	75.4
-1	1	-1	-1	-1	1	-1	1	1	0	1	1	118.6
1	-1	-1	1	1	1	-1	1	-1	-1	0	-1	124.0
-1	1	1	-1	-1	-1	1	-1	1	1	0	1	118.6
1	1	-1	-1	1	1	1	-1	1	-1	-1	0	118.6
-1	-1	1	1	-1	-1	-1	1	-1	1	1	0	29.8
1	1	1	-1	-1	1	1	1	-1	1	-1	-1	78.6
-1	-1	-1	1	1	-1	-1	-1	1	-1	1	1	119.3
1	1	1	1	-1	-1	1	1	1	-1	1	-1	26.7
-1	-1	-1	-1	1	1	-1	-1	-1	1	-1	1	122.4
1	-1	1	1	1	-1	-1	1	1	1	-1	1	71.2
-1	1	-1	-1	-1	1	1	-1	-1	-1	1	-1	63.8
1	1	-1	1	1	1	-1	-1	1	1	1	-1	40.4
-1	-1	1	-1	-1	-1	1	1	-1	-1	-1	1	118.6
1	-1	1	-1	1	1	1	-1	-1	1	1	1	118.6
-1	1	-1	1	-1	-1	-1	1	1	-1	-1	-1	119.3
1	-1	-1	1	-1	1	1	1	-1	-1	1	1	118.6
-1	1	1	-1	1	-1	-1	-1	1	1	-1	-1	101.9
0	0	0	0	0	0	0	0	0	0	0	0	35.1

B. Detector schematics

Plexiglass/Wood version:



Cardboard Version:



Additional files available at https://gmodetective.com/resources/ and https://github.com/MakerLabCRI/GMODetective-Detector

C. Hardware and Wetware Guides

GMO Detective 

Hardware Guide:

The Hardware guide will help you to gather, buy and build all the materials you need to do the experiments. This consists of reusable devices such as the open hardware fluorescence detector and also consumables which are used with each experiment.

Reusables **1**

Makabales 1

Open Hardware Detector 1

Holders 2

Buyable or makeable or hackable 3

Common materials anyone probably has: 3

Stable heating: 3

20µµL Pipettes 3

Consumables **4**

Reusables

Makabales

Files for PCB and Laser cutter:

https://gmodetective.com/wp-content/uploads/2018/10/GMOdetective_Hardware_FilesV0.1.zip

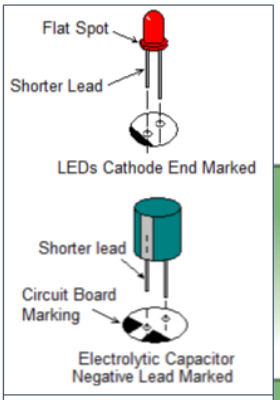
a. Open Hardware Detector

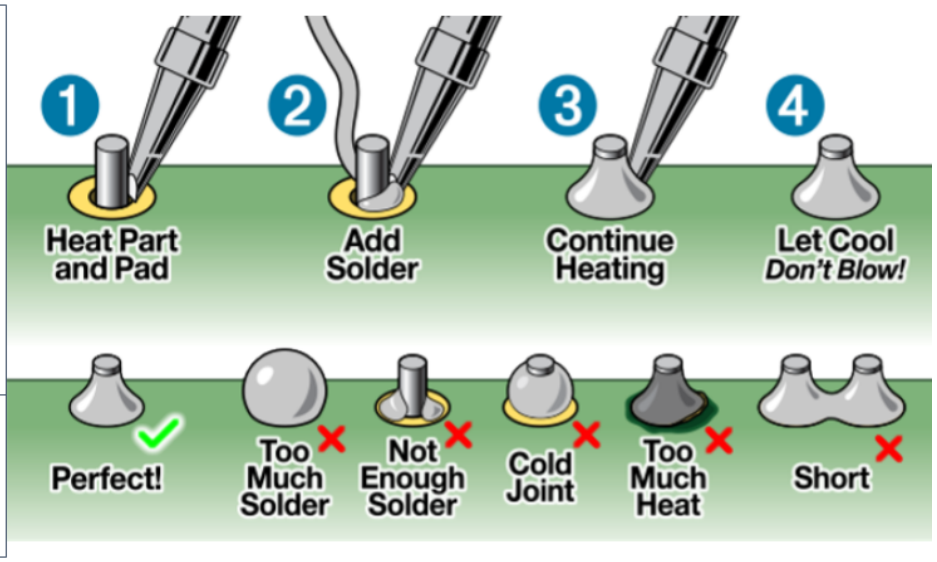
This is a cheap DIY fluorescence detector. It is easy to build and use. A buyable version should be available soon. You will need to know how to solder as well as a soldering iron, 15x15cm 2/3mm plexiglass/wood/foldable cardboard, a laser cutter and the following parts:

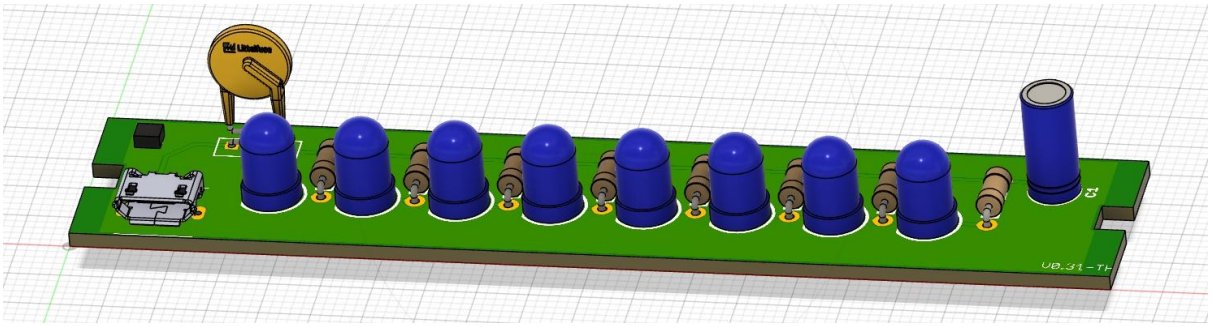
1xPCB	https://www.pcbway.com The file is in eagle format (free for students) We can also send you some :)
8x5mm blue LEDs 470nm	https://www.mouser.com/__/?Keyword=C503B-BAN-CZ0A0451
8x68/100 ohms resistors	https://www.mouser.com/__/?Keyword=CFR-25JT-52-68R
1xCapacitor 10 µF	https://www.mouser.com/__/?Keyword=ECA-1JM100B
1xFuse 0,5 A	https://www.mouser.com/__/?Keyword=RXEF025
1xMicro USB Connector 90°	https://www.aliexpress.com/item/1005001515820458.html

Orange Filters	179 Chrome Orange ; http://www.leefilters.com/lighting/colour-details.html#179
Blue Filter	716 Mikkel Blue : http://www.leefilters.com/lighting/colour-details.html#716

Solder the parts onto the PCB: (**Careful LEDs and Capacitor are polar, i.e. have a direction the other components do not, this can also be seen by the different lengths of the legs**)



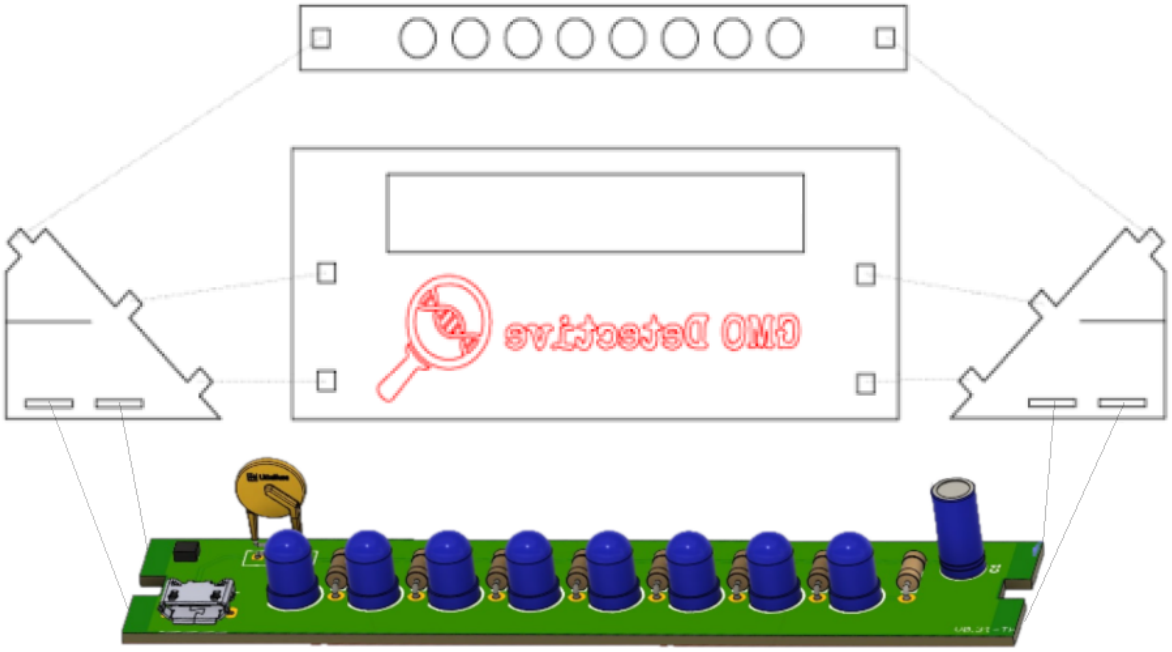


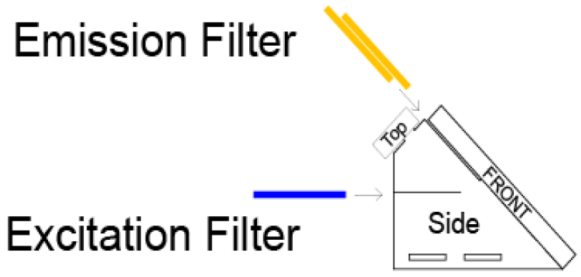


Laser-cut the legs tube holder and front panel

Slide the PCB into the holes made for it in the two leg parts, with the USB port facing the back (flat) part **NOT** the angled part (front)

Then attach the front panel and top tube holder.





Slide one 1.5x11cm blue filter into leg/side slits, and **2** orange filters into the smale space between the legs/sides and the front panel so you see orange through the view hole!

!!!The LEDs are very bright, try to not look directly at them!!!

Connect to a 5v power source (USB).

b. Holders

Laser-cut the legs and tube holder and connect

Buyable or makeable or hackable

Common materials anyone probably has:

You will need a mug or beaker to hold boiling water for 5 minutes during the sample prep, anything that can hold coffee or tea will do.

Aluminum foil to hold the tubes in place.

Permanent waterproof markers too, so you know what is what :)

Stable heating:

We need something that can keep a stable 63℃

If you have a PCR machine/heat block/Scientific water bath then these will all be perfect.

Otherwise these portable sous vide vacuum cookers are very precise and affordable, additionally you can also experiment with new ways of cooking!

https://www.aliexpress.com/item/Vacuum-Slow-Sous-Vide-Precision-Food-Cooker-1500W-Powerful-Immersion-Circulator-LCD-Digital-Timer-Display-Stainless/32869232621.html?spm=a2g0s.8937460.0.0.302d2e0eDrMWuV

Or

https://anovaculinary.com/

Or

You can also make one yourself!

https://www.instructables.com/howto/sous+vide/

https://www.instructables.com/id/DIY-Sous-Vide-Machines/

2-20µL Micropipette

Buy:

https://www.aliexpress.com/item/2-20ul-Single-Channel-Adjustable-Micro-Pipette-Transfer-Pipette-Lab-Pipettor-Buy-1-Pipette-Give-50pcs/32814661287.html

Consumables

These you will need to buy. Every experiment needs:

~4 1.5mL tubes

1 PCR strip

2 Pestles

2 gloves

And ~10 tips

1,5 mL microtubes	https://www.aliexpress.com/item/1-5ml-Clear-plastic-centrifugal-test-tube-Sample-vial-with-snap-cap-for-samples-food-colourings/32694400420.html
PCR 8 tube Strips	https://www.aliexpress.com/item/200PCS-4ml-Centrifuge-Bottles-Test-Tube-With-Lid-Calibration-Round-Bottom-Seed-Container-Laboratory-Supplies-1500r/32708099906.html
micropestles	https://www.aliexpress.com/item/50-pcs-lot-5mm-70mm-Polypropylene-Pestle-grinding-PP-rod-tip-for-1-5ml-centrifuge-tube/32762070308.html
Pipet Tips	https://www.aliexpress.com/item/New-200ul-PP-Plastic-Pipette-Tips-For-Microbiological-Test-Pipettor-Yellow-Pipet-Tips-Disposable-Pipette-Tip/32523839777.html
Pipet Tips in Box	https://www.aliexpress.com/item/Free-shipping-Plastic-Pipette-box-96vents-box-96pcs-200ul-yellow-pipette-tips/32595675105.html
gloves	https://www.aliexpress.com/item/3-Sizes-High-Quality-1box-A-lot-powder-free-Nitrile-Disposable-glove-latex-Gloves-non/32845058449.html

GMO Detective



Wetware Guide:

The wetware guide will help you to gather and buy all the biological (wet) materials you need to set up the experiments. This consists of reagents and DNA (controls and primers). These companies usually do not sell to private individuals and the prices go down drastically when buying in bulk, so better to contact us :)

1. **Enzyme mix** **1**
2. **Primers** **2**
   3. COX1 : Plant Gene primers 3
   4. GMO Gene primers 3
5. **Making the mastermix** **6**

1. Enzyme mix

This is a premade mix consisting of an improved Isothermal Strand Displacing DNA polymerase enzyme along with Nucleotides and a buffer that allows the reaction to happen, all you need to add to this is a primer set and sample.

For simplicity use NEB WarmStart® LAMP Kit (DNA & RNA) E1700:

https://www.neb.com/products/e1700-warmstart-lamp-kit-dna-rna

Available worldwide through their Subsidiaries / Distributors.

The small package is enough for 250 reactions which is ~30 Experiments.

(a regular LAMP reaction is 25ul, we do 8x10ul reactions per experiment)

For a larger scale or more affordability, you can buy the reagents separately (Bst 2.0 Warmstart polymerase (Or make your own see https://www.medrxiv.org/content/medrxiv/early/2020/10/05/2020.10.02.20203356.full.pdf and https://reclone.org/), dNTPs, MgSO4, Isothermal amplification buffer) or as pre-made freeze dried tubes including everything from us (see contact info on website) .

2. Primers

The experiment uses 2 sets of QUASR LAMP primers sets specific to a certain region. Each set has 7 Primers :

2 long internal primers (FIP/BIP)

2 short external primers (F3/B3)

2 loop primers to make the reaction faster (LF/LB)

1 quenching primer to quench the fluorescence if the reaction is negative (FIP/BIP-Q)

Primers can be ordered from your local supplier, but are cheaper in bulk so contact us for purchasing together :)

Standard desalination is fine, except for quencher probes which need to be HPLC purified

Idtdna.com

Eurofinsgenomics.com

Thermofisher.com

Sigmaaldrich.com

a. COX1 : Plant Gene primers

COX1 gene common to all plants

Based on: Rapid Detection of Phytophthora ramorum and P. kernoviae by Two-Minute DNA Extraction Followed by Isothermal Amplification and Amplicon Detection by Generic Lateral Flow Device J. A. Tomlinson, M. J. Dickinson, and N. Boonham
doi:10.1094/ PHYTO-100-2-0143

Primer	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 or Iowa Black® FQ

b. GMO Gene primers

CaMV-35S promoter common to most GMO plants:

Based on: Zahradnik Celine, Kolm Claudia, Martzy Roland, Mach Robert L., Krska Rudolf, Farnleitner Andreas H., Brunner Kurt. Detection of the 35S promoter in transgenic maize via various isothermal amplification techniques: a practical approach. Analytical and Bioanalytical Chemistry. 2014;406(27):6835–6842.
doi: 10.1007/s00216-014-7889-2.

Primer	Sequence 5'to3'	Modification
35SZ_FIP	acgtggttggaacgtcttcttcccaaagatggaccccca	
35SZ_BIP	atctccactgacgtaagggatgatagaggaagggtcttgcga	5' FAM
35SZ_F3	aagatgcctctgccgaca	
35SZ_B3	cagcgtgtcctctccaaat	
35SZ_FLoop	tccacgatgctcctcgtg	
35SZ_BLoop	acgcacaatcccactatcct	
35SZ_BIPQ	C AGT GGA GAT	3' Black Hole Quencher®-1 Or Iowa Black® FQ

In each 10ul reaction in a PCR tube we have in the end:

5ul LAMP reaction mix

3ul Primer mix

2ul sample

=10ul

Guide to 3x Primer mix dilutions (starting from 50mM Primer stock) for different numbers of reactions (for 100 mM stock halve the volumes of primers and add appropriate volume of water instead.)

	Primer	Primer working stock conc.	Primer conc. in 10µl LAMP rxn	Vol. of primer (1 rxn) µL	Vol. of primer (10 rxn) µL	Vol. of primer (50 rxn) µL	Vol. of primer (100 rxn) µL	Vol. of primer (200 rxn) µL	Vol. of primer (500 rxn) µL
	FIP	50mM	1.6µM	0.32	3.2	16	32	64	160
	BIP	50mM	1.6µM	0.32	3.2	16	32	64	160
	F3	50mM	0.2µM	0.04	0.4	2	4	8	20
	B3	50mM	0.2µM	0.04	0.4	2	4	8	20
	FLP	50mM	0.8µM	0.16	1.6	8	16	32	80
	BLP	50mM	0.8µM	0.16	1.6	8	16	32	80
	Quencher	50mM	2.4µM	0.48	4.8	24	48	96	240
	DNaseFree H2O			1.48	14.8	74	148	296	740
	Final volume			3	30	150	300	600	1500
Semi opt	Primer	Primer working stock conc.	Primer conc. in 10µl LAMP rxn	Vol. of primer (1 rxn) µL	Vol. of primer (10 rxn) µL	Vol. of primer (50 rxn) µL	Vol. of primer (100 rxn) µL	Vol. of primer (200 rxn) µL	Vol. of primer (500 rxn) µL
	FIP	50mM	1.2µM	0.24	2.4	12	24	48	120
	BIP	50mM	1.2µM	0.24	2.4	12	24	48	120
	F3	50mM	0.2µM	0.04	0.4	2	4	8	20

	B3	50mM	0.2µM	0.04	0.4	2	4	8	20
	FLP	50mM	1µM	0.2	2	10	20	40	100
	BLP	50mM	1µM	0.2	2	10	20	40	100
	Quencher	50mM	1.6µM	0.32	3.2	16	32	64	160
	DNaseFree H2O			1.72	17.2	86	172	344	860
	Final volume			3	30	150	300	600	1500

3. Making the mastermix

We want to make enough mastermix for **X** experiments

Each experiment is 8 tubes, 4 for plant the gene and 4 for the GMO gene.

We mix 5µl*4***X** LAMP mix with 3µl*4***X** Primer mix for each primer set.

It is better to usually make about 10% extra just in case of mistakes and spilling

Enjoy!

Lyophilization (Manufacturing) Protocol:

Also available at:

https://protocols.io/view/freeze-drying-lyophilization-of-gmodetective-exper-bkvtkw6n



Freeze-drying (Lyophilization) of GMODetective experiments

Guy Aidelberg[1]

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Guy Aidelberg
CRI (Center for research and interdisciplinarity) Paris

ABSTRACT

A protocol for freeze-drying (Lyophilization) of GMODetective experiments, and more generally any LAMP experiment

The amounts here are for one 96 well standard PCR plate and for **10 µl** reactions

This can be scaled for any amount and size.

PROTOCOL INFO

Guy Aidelberg . Freeze-drying (Lyophilization) of GMODetective experiments. **protocols.io** https://protocols.io/view/freeze-drying-lyophilization-of-gmodetective-exper-bkvtkw6n

KEYWORDS

LAMP, GMO, Education, Open Science

CREATED

Sep 04, 2020

LAST MODIFIED

Sep 05, 2020

PROTOCOL INTEGER ID

41619

GUIDELINES

The amounts here are for one standard 96 well PCR plate (12 experiments), and for **10 µl** reactions

This can be scaled for any amount of experiments at any reaction volume.

MATERIALS

NAME	CATALOG #	VENDOR
Isothermal Amplification Buffer - 6.0 ml	B0537S	New England Biolabs
Magnesium Sulfate (MgSO4) Solution - 6.0 ml	B1003S	New England Biolabs
Deoxynucleotide (dNTP) Solution Mix	N0447S	New England Biolabs
Bst 2.0 Warm Start DNA Polymerase Glycerol-free	M0402B	New England Biolabs
D-( )-Trehalose dihydrate	T5251	Sigma Aldrich

EQUIPMENT

NAME	CATALOG #	VENDOR
Scientific Freeze Dryer	HRFD-Med-Sci-EU	Harvest Right

SAFETY WARNINGS

Standard Polymerases usually come in 50% Glycerol, so as to be stable in the **-20 °C** freezer. The glycerol interferes with freeze-drying, make sure your enzyme(s) is/are Glycerol free and stored at **-80 °C** .

BEFORE STARTING

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

1 Thaw vortex and spindown dNTPs, Primer mixes, MgSO4, Isothermal amplification buffer and Enzyme.

2 In 2 separate Eppendorf tubes (one for each primer set) mix together **77 µl dNTPs**
10 Milimolar (mM) each dNTP + **55 µl of each 10x Primer Mix** +
1.5 µl Glycerol free Bst 2.0 WarmStart® DNA Polymerase **120000 U/ml** + **110 µl Trehalose**
50 Mass Percent (for a 50% trehalose solution, mix 500mg trehalose with 1mL H20) +
196.5 µl DNAse free water Vortex all or mix by pipetting up and down and then spindown.

2.1 Either now or at a later time create the rehydration buffer. For a whole plate mix
100 µl 10X Isothermal Amplification Buffer +
60 µl Magnesium Sulfate (MgSO4) Solution + **640 µl DNAse free water**

3 In the first 4 rows (A-D) of a PCR plate place **8 µl** of the Plant(COX) master mix
In the second 4 rows (E-H) of a PCR plate place **8 µl** of the GMO(35S) master mix

3.1 Optionally add **2 µl** of the positive controls to each tube in rows B and F

4 Seal the plate(s) with either foil or parafilm and then puncture the seal. This is so when the reactions are freeze-dried the small pellets do not "jump" out of the tubes. A 96-Pin Replicator is perfect for this but a multi-pipette toothpick scalpel or scissors will do.

5 Depending on your freeze drier, you might need to now freeze the tubes, and make sure they remain frozen (such as by placing in a frozen metal rack or touching a frozen metal block).
Otherwise, simply place in a freeze-drier and run overnight or until done.
I start by freezing to ~ **-40 °C** for a few hours then turn on the vacuum (aiming for 500mtorr) for another couple of hours, then slowly heating by **10 °C** every hour with the vacuum still on.

Scientific Freeze Dryer
lyophilizer
Harvest Right HRFD-Med-Sci-EU



6 Make sure each tube has a similarly sized dried pellet and reseal the plate.
Store in a dark and dry place preferably in a sealed bag with a desiccant.

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D. Materials and Prices

Prices here are calculated for small to medium scale production and can rapidly go down with quantity/bulk purchasing

For 1 8-Strip tube (Experiment) Reagents Purchased from NEB and Primers from IDT

Type	Name	Standard LAMP MasterMix	GMO Detective	Optimized
WetWare	Enzyme	€16.00	€0.97	€0.61
	MgSO4		€0.02	€0.02
	dNTPs		€0.36	€0.41
	Buffer		€0.03	€0.03
	Primers	€0.65	€0.26	€0.19
Total		€16.65	€1.65	€1.27

Consumables:

Prices here are for buying from aliexpress, usually with free worldwide shipping but can take up to 2 months. For links see hardware/material guide. Similar materials can be bought from local scientific suppliers such as thermofisher.com or sigmaaldrich.com, but cost ~1.5-5x more. Local Amazon usually also has all needed materials at a middle price.

.

Type	Name	For 1 Workshop (16 ppl)	# Needed for 1 experiment	Aliexpress		For 1 Workshop (16 ppl)	For 1 Experiment
				# of pieces/ lot	Price		
Consumable	1,5 mL microube	40	4	100	€2.10	€0.84	€0.08
	PCR 8 tube strip	10	1	200	€17.06	€0.85	€0.09
	Pestles	20	2	1000	€64.55	€1.29	€0.13
	Pipet Tips	100	10	1000	€9.07	€0.91	€0.09
	Pipet Tip in Box	4	1	1	€3.98		
	Gloves	20	1	50	€8.00	€3.20	€0.16
	Aluminium foil	1	0.1	30	€1.75	€0.06	€0.01
					Total	€7.15	€0.55

Reusable:

Detectors: Prices here are for an unbuilt detector (parts) in Europe at small to medium scale (~100), this goes down drastically if going to larger scales (thousands) and if purchasing components directly in China. Similar prices could be achieved for premade (soldiered) detectors if using SMD (Surface Mount Device) components which are smaller, more affordable, don't require drilling in the PCB and allow for automation (this has been tested by me but data not shown)

Type	Name	For 1 Work-shop (16 ppl)	# for 1 detector	Price /unit	For 1 detector	For 8 detectors
Electronic component	PCB (Printed Circuit Board)	8	1	€0.20	€0.20	€1.60
	LEDs 468 nm	70	8	€0.15	€1.20	€9.60
	Resistor 68 ohms	70	8	€0.01	€0.08	€0.64
	Capacitor 10 µF	10	1	€0.05	€0.05	€0.40
	Fuse 0,5 A	10	1	€0.20	€0.20	€1.60
	USB connector	10	1	€0.02	€0.02	€0.20
Consumable	Solder	1	0.05	€2.93	€0.15	€1.17
Plastics	3mm plexiglass	400cm2	120x82mm	€7.53	€0.03	€0.22
	Orange filters	400x110mm	40x110mm	€6.14	€0.05	€0.36
	Blue filters	150x110mm	15x110mm	€6.14	€0.02	€0.14
				Total	€1.97	€15.73

Other reusables :
Heat source :Depends on what you have and need, a sous vide precision cooker that you can also cook with for 60-200$, or a professional heat block/water-bath/pcr machine 200- 5000$
Pipette:
2-20ul Single Channel Adjustable MicroPipette Transfer Pipette Lab Pipettor 1 €20.36
https://www.aliexpress.com/item/2-20ul-Single-Channel-Adjustable-Micro-Pipette-Transfer-Pipette-Lab-Pipettor-Buy-1-Pipette-Give-50pcs/32814661287.html

Corning® Lambda® plus single channel pipettor 1 €171.77
https://www.sigmaaldrich.com/catalog/product/sigma/cls4072?lang=fr&region=FR&cm_sp=Insite-_-prodRecCold_xviews-_-prodRecCold5-2

Fine tip permanent markers, a source of boiling water, and a cup/mug/glass to put it in.

E. Extended Teacher/Instructor Guide

Quick start guide
GMO Detective

Materials **2**
- Materials provided: 2
- Materials not provided that you will need: 2

Purpose & Principle of the experiments **3**
- Starting from a question 3
- Extracting DNA from our samples 3
- Testing for the presence of GMO DNA 4
- Mounting the fluorescence detector 5
- Interpreting the results 6

Protocol: **7**

Deployment in an educational setting **9**

You will also find in the attached files (in HD) :

- The protocol
- The guide for using a pipette
- The Fluorescence Detector Mounting Guide
- The help sheet for interpreting the results
- The theoretical framework and detailed explanation of this experiment

These documents are helpful for classroom use (e.g., to print 15 copies of the protocol) or to understand the underlying scientific principles

Materials

Materials provided:

- 1 x 8 Tube PCR strip containing freeze-dried(lyophilized) reagents
    - Tubes 1 to 4 (green) : Primers for Plant Gene + Enzyme + dNTPs
    - Tube 5 to 8 (blue) : Primers for GMO marker + Enzyme + dNTPs
- 1 x DNAse-free water tube
- 1 x Tube containing rehydration and reaction buffer solution
- 2 x Empty 1.5mL Eppendorf tubes
- 2 x Small pestles
- The fluorescence detector

Materials not provided that you will need:

- 1 x Water bath set to 63°C (or sous vide precision cooker or PCR machine)
- 1 x Micropipette 1-10 or 2-20
- 8 x Appropriate tips for the above micropipette
- Boiling water source
- Aluminum foil
- Cups/mugs for the boiling water
- Thin tipped permanent markers

For your information, the volumes to be handled are 2µL, 8µL, and 60µL (1x60µL, 3x20µL, or 6x10µL). The micropipette can be replaced by any other tool able to transfer these same precise volumes.

Purpose & Principle of the experiments

Aim of the experiment: to look for the presence of **GMO** corn or soybean **DNA** in a food sample.

Principle of the experiment :

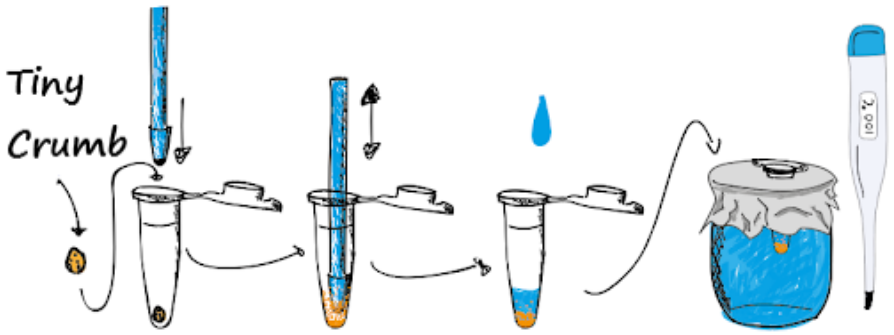
1) Starting from a question

Many great experiments start with a precise question. Here the question is whether a food (corn or soy) contains a transgenic element(is GMO).

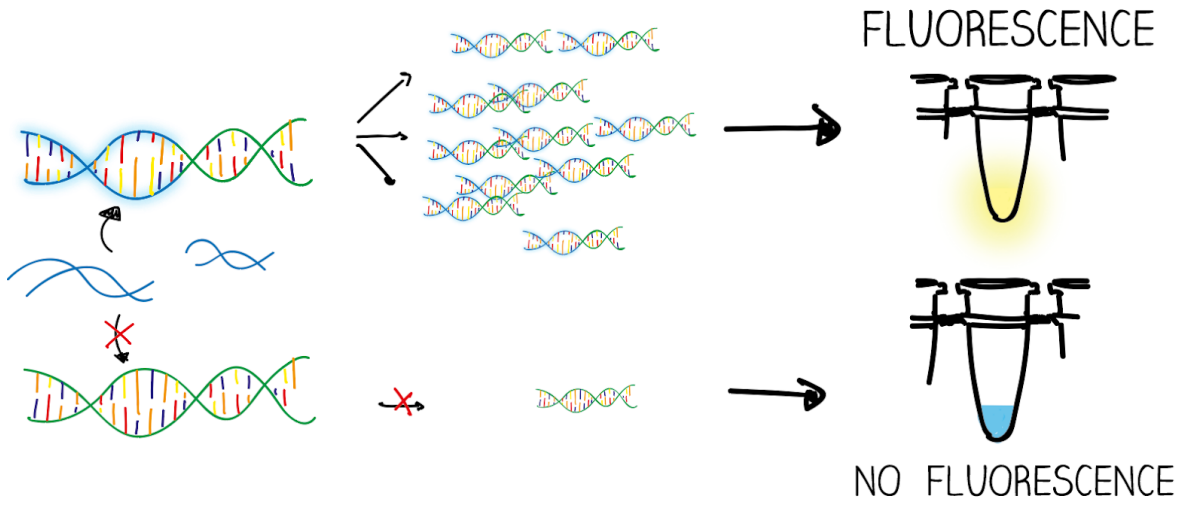


2) Extracting DNA from our samples

To find out if our food contains GMOs we will look for the presence of specific DNA sequences commonly used in plant genetic modifications. A crucial step is therefore the extraction of the DNA from the food. This extraction is carried out by grinding and then heating.



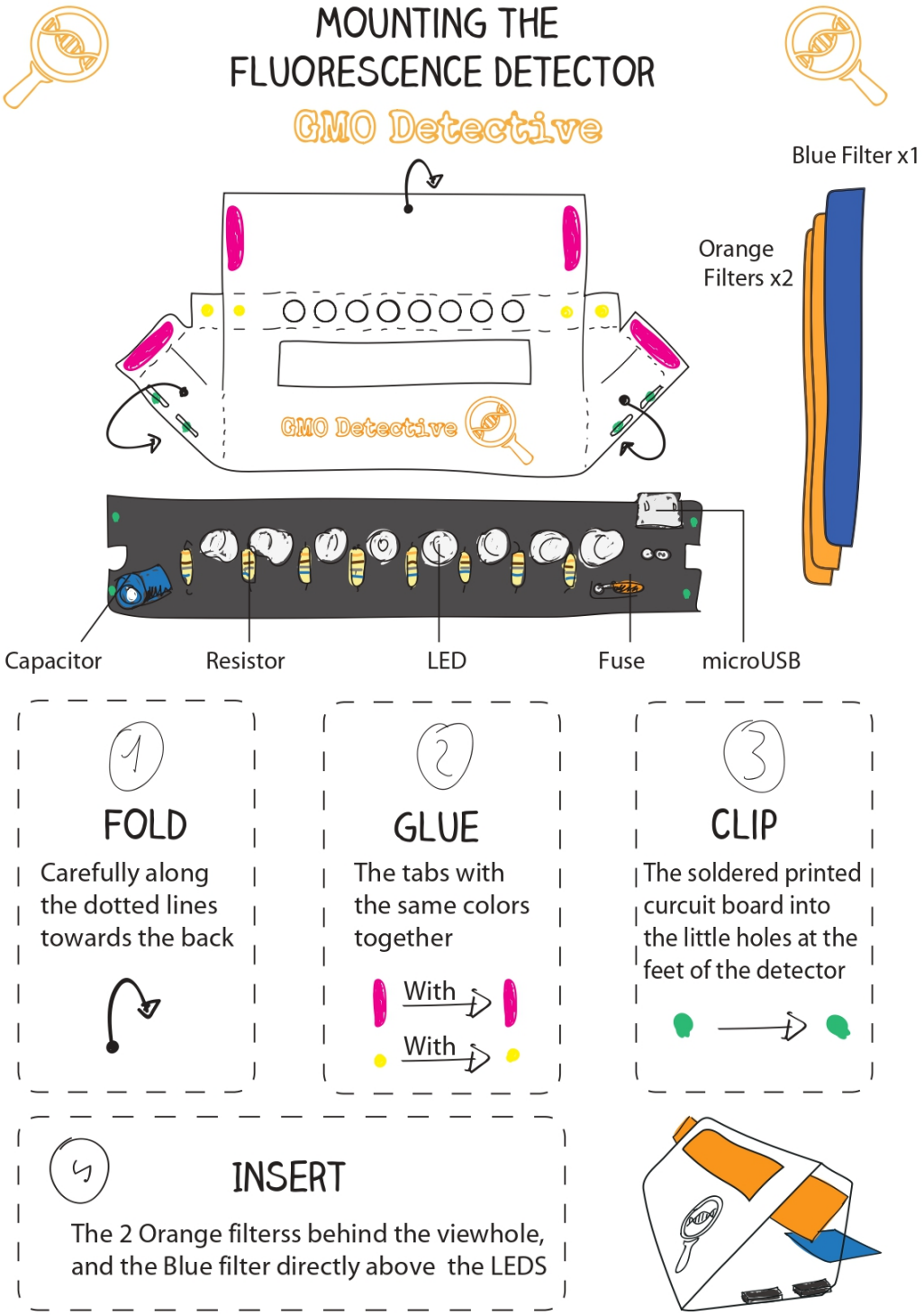
3) Testing for the presence of GMO DNA



Next, we use a **DNA amplification technique** called **LAMP**(**L**oop-mediated-isothermal **Amp**lification). The primers used in these reactions are directed either to a **genetic marker** universal to **all plants** (serving as a control for successful extraction of DNA) or to a genetic marker (CaMV-P35s; indicating the presence of GMOs in our sample) typically present in **GMO plants** (shown above). If the target is present, there will be massive and rapid amplification of DNA. If the marker is absent, there will be no amplification. The results are finally observed by **fluorescence** thanks to the presence of a **fluorophore**: fluorescein (FAM).

For more information on the theoretical basis behind the highlighted concepts and the theoretical foundations used for this experiment, please refer to the appendix.

4) Mounting the fluorescence detector



5) Interpreting the results

Seeing the results with your eyes is easy, but Interpreting them is slightly more complicated. Understanding exactly what is in each tube and its purpose will help.

INTERPRETING THE RESULTS

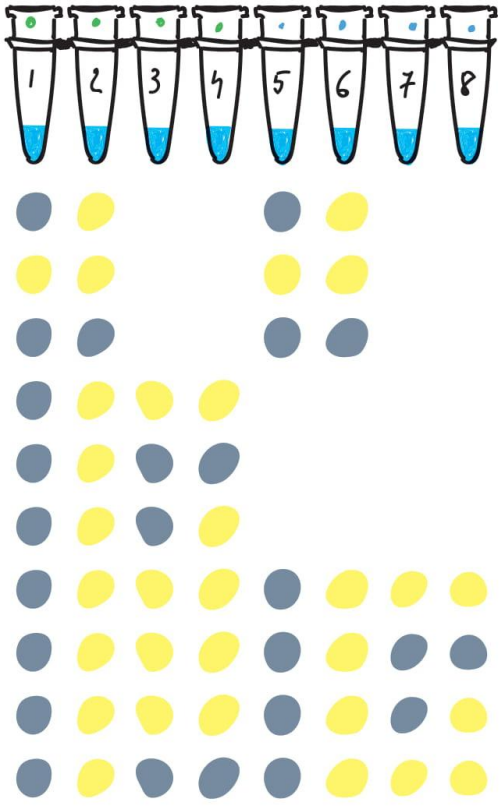
GMO Detective

THIS SHEET IS A TOOL TO HELP YOU INTERPRET AND UNDERSTAND YOUR RESULTS.



In this example, the controls work perfectly, the negatives are negative, the positives are positive, the DNA from both samples have been properly extracted, sample 1 does not contain GMOs while sample 2 does.

1 - NEGATIVE CONTROL PLANT DNA: VERIFIES THE ABSENCE OF CONTAMINATION IN THE 'PLANT' REACTION MIX.
2 - POSITIVE CONTROL PLANT DNA: VERIFIES THAT THE REAGENTS IN THE 'PLANT' REACTION MIX ARE WORKING AS INTENDED.
3 - TEST SAMPLE 1 PLANT DNA: TESTS FOR THE PRESENCE OF PLANT DNA - VERIFIES THAT THE EXTRACTION WORKED.
4 - TEST SAMPLE 2 PLANT DNA: TESTS FOR THE PRESENCE OF PLANT DNA - VERIFIES THAT THE EXTRACTION WORKED.
5 - NEGATIVE CONTROL GMO DNA: VERIFIES THE ABSENCE OF CONTAMINATION IN THE 'GMO' REACTION MIX.
6 - POSITIVE CONTROL GMO DNA: VERIFIES THAT THE REAGENTS IN THE 'GMO' REACTION MIX ARE WORKING AS INTENDED.
7 - TEST SAMPLE 1 GMO DNA: TESTS FOR THE PRESENCE OF GENETICALLY MODIFIED PLANT DNA IN SAMPLE 1.
8 - TEST SAMPLE 2 GMO DNA: TESTS FOR THE PRESENCE OF GENETICALLY MODIFIED PLANT DNA IN SAMPLE 2.



GUIDE TO INTERPRETING THE RESULTS

Negative result Positive result

OK: NEGATIVE CONTROLS NEGATIVE, POSITIVE CONTROLS ARE POSITIVE

/!\ NEGATIVE CONTROLS ARE POSITIVE: POSSIBLE CONTAMINATION

/!\ POSITIVE CONTROLS ARE NEGATIVE: REAGENTS/HEATING PROBLEM

OK: PLANT DNA IS DETECTED: EXTRACTION WORKED WELL

/!\ NO PLANT DNA IS DETECTED: EXTRACTION OR SAMPLE PROBLEM

/!\ EXTRACTION SAMPLE 1 OK; EXTRACTION SAMPLE 2 PROBLEM

OK: BOTH SAMPLES CONTAIN GMOS

OK: BOTH SAMPLES DO NOT CONTAIN GMOS

OK: ONLY SAMPLE 2 CONTAINS GMOS

/!\ INCONCLUSIVE; HYPOTHESIS: SAMPLES NOT ADDED IN PLANT TUBES

Protocol:

PART I - DNA EXTRACTION

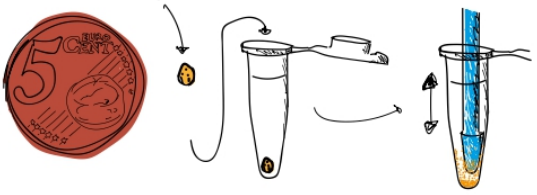
GMO Detective

THE FIRST STEP IN DETERMINING IF THERE MIGHT BE GMO DNA IN YOUR SAMPLES IS TO EXTRACT THE DNA FROM THE PLANT CELLS CONTAINED IN THE FOOD. THE EXPLANATIONS BELOW SHOWS YOU HOW TO PROCEED, BUT BEFORE YOU BEGIN, HERE ARE TWO RULES OF THUMB:

1 - BEWARE OF CONTAMINATIONS: KEEP YOUR HANDS CLEAN, ALWAYS USE CLEAN PESTLES, ALWAYS USE CLEAN TIPS, ETC.
2 - ALWAYS LABEL YOUR TUBES (YOUR NAME & CONTENT) WITH A PERMENANT MARKER



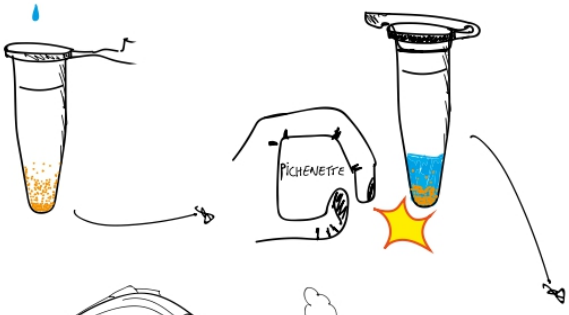
1. TAKE ABOUT 0.005 GRAMS (1-2MM NOT MORE) OF THE FOOD YOU WANT TO TEST, THEN PLACE IT IN AN EMPTY MICROTUBE TUBE. LABEL IT !!!! YOU MUST HAVE TWO EPPENDORF TUBES, ONE FOR EACH SAMPLE.
FOR YOUR INFORMATION, 0.005 GRAM IS PRETTY MUCH LIKE THAT. (1-2 MM)



2. USING THE SMALL PESTLES, THOROUGHLY GRIND / CRUSH EACH OF YOUR SAMPLES INSIDE THE MICROTUBE. ONE PESTLE PER SAMPLE !!



3. USING A MICROPIPETTE WITH A CLEAN TIP, ADD 60 µL OF DISTILLED WATER (DNASE FREE) TO EACH MICROTUBE CONTAINING A SAMPLE. DEPENDING ON YOUR AVCAILABLE PIPETTES AND TIPS YOU CAN DO 3X20 µL OR 1X60 µL.



4. CLOSE THE CAPS OF THE MICROTUBES AND MIX BY TAPPING THE BOTTOM OF THE TUBE OR WITH A VORTEX

5. BOIL WATER AND POUR IT INTO AN APPROPRIATE CONTAINER (BEAKER, GLASS, MUG, ETC.)





6. COVER THE CONTAINER FILLED WITH BOILING WATER WITH ALUMINUM FOIL.

7. PUSH YOUR EPPENDORF TUBES THROUGH THE ALUMINUM FOIL, MAKE SURE THE MATERIAL IS FULLY IMMERSED IN THE WATER



8. WAIT 5 MINUTES BEFORE REMOVING THE TUBES, YOU CAN START READING THE SECOND PART NOW :)

PART II - SPECIFIC DNA AMPLIFICATION

GMO Detective

THE SECOND PART IS TO AMPLIFY THE DNA EXTRACTED IN STEP I. REMENER WE ARE LOOKING FOR 2 DIFFERENT GENES, A COMMON PLANT GENE TO MAKE SURE WE EXTRACTED THE DNA AND A COMMON GMO GENE. KEEP IN MIND THE TWO GOLDEN RULES:

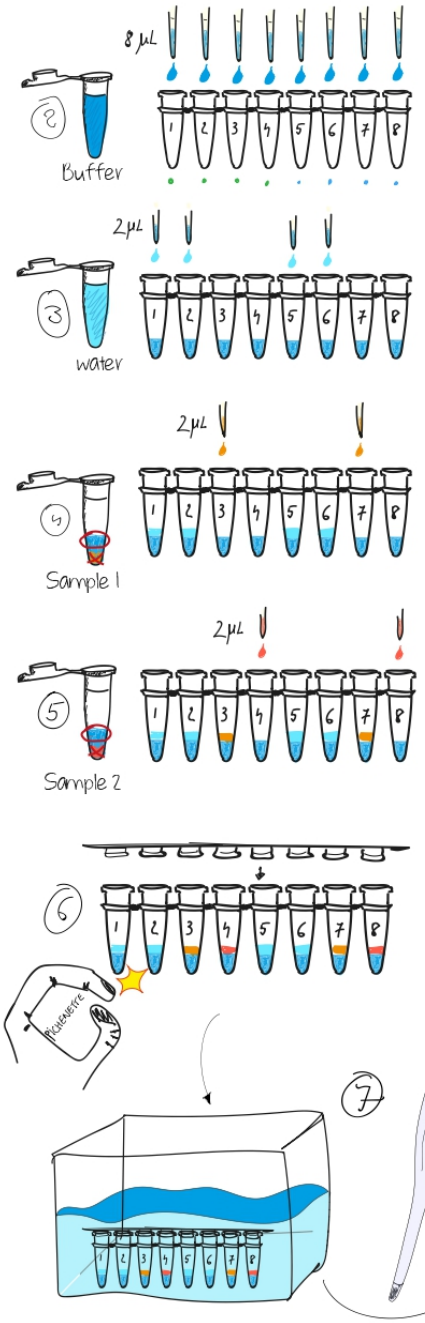
1 - BEWARE OF CONTAMINATIONS: KEEP YOUR HANDS CLEAN, ALWAYS USE CLEAN TIPS, DONT TOUCH THE CAPS, ETC.
2 - ALWAYS LABEL THE TUBES WITH A PERMENANT MARKER TO REMEMBER WHAT IS WHAT!

1 - NEGATIVE CONTROL PLANT DNA
2 - POSITIVE CONTROL PLANT DNA
3 - TEST SAMPLE 1 PLANT DNA
4 - TEST SAMPLE 2 PLANT DNA

DETECTS PLANT DNA TO SEE IF DNA EXTRACTION HAS SUCEEDED

5 - NEGATIVE CONTROL GMO DNA
6 - POSITIVE CONTROL GMO DNA
7 - TEST SAMPLE 1GMO DNA
8 - TEST SAMPLE 2 GMO DNA

DETECTS THE PRESENCE OF A COMMON ELEMENT IN GENETICALLY MODIFIED CORN OR SOYBEAN DNA.



1. TAKE CARE OF THE STRIP OF 8 PCR TUBES. EVEN IF THEY LOOK EMPTY, THESE TUBES CONTAIN ALL THE FREEZE DRIED REAGENTS YOU NEED. MARK YOUR NAME/INITIALS AND NUMBER THE TUBES AND CAPS
2. RESUSPEND THE CONTENTS OF THE TUBES BY ADDING 8µL BUFFER INTO EACH OF THE 8 TUBES
3. ADD 2 µL OF CLEAN (DNASE FREE) WATER TO THE NEGATIVE CONTROL TUBES (1 & 5) AND TO THE POSITIVE CONTROL TUBES (2 & 6)
4. ADD 2 µL OF EXTRACTED DNA FROM SAMPLE 1 TO TUBES 3 AND 7
5. ADD 2 µL OF EXTRACTED DNA FROM SAMPLE 2 TO TUBES 4 AND 8
6. CLOSE THE 8 TUBES WELL! AND MIX BY TAPPING OR VORTEXING
7. INCUBATE AT ~63 ° C FOR ~1 HOUR
8. REMOVE FROM INCUBATION MAKE SURE THEY ARE COOL AND THEN PLACE THE TUBES IN THE FLUORESCENCE DETECTOR TO VIEW THE RESULTS

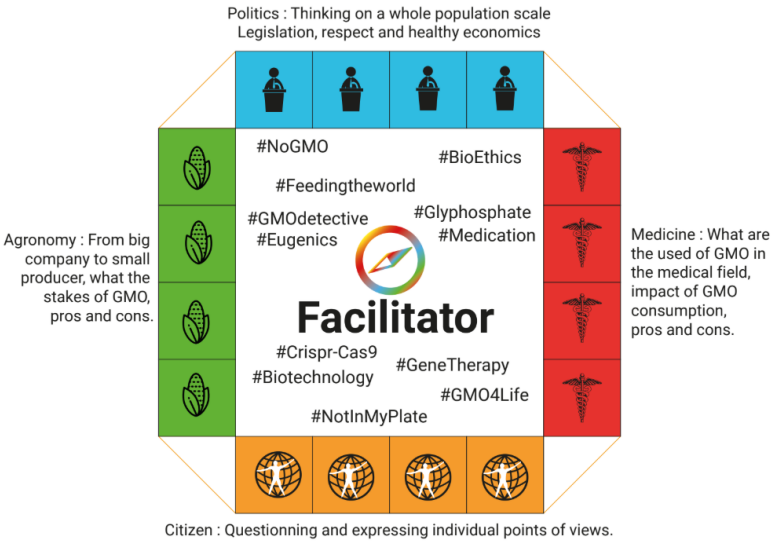
Deployment in an educational setting

This GMO kit has been thoroughly tested and highly praised with students (and their teacher) between the ages of 14 and 19. The implementation of this experiment is simple enough to be carried out in class, the main thing is to link the hands-on experiment to the concepts of genetics and GMOs that will enable insight and comprehension.
It is possible to carry out this experiment with families or with younger children, as it is still fun and understandable even without going deeply into the details of the mechanisms related to DNA amplification.

For the hands-on part, the most difficult part is the precise measurement of volumes. It is important to choose the right tools and make sure that the students know how to pipette. If this is the first time it is better to have them practice first using only water. A guide to using micropipettes is available in the appendix

In class, we suggest the following lesson sequence:

Session #1	What is DNA? What are genes? How do they change move and evolve?
Session #2	What are GMOs? What can they be used for? In which fields?
Session #3	Transdisciplinarity in Biology: Fluorescence, DIY/T, and GMO detection.
Session #4	Hands-on use of the kit and experimentation.
Session #5	A debate about GMOs and their use, using a role-playing game (see below for roles and layout)



F. Questionnaires

Before English:

GMO Workshop before Questionnaire:

Age :

Gender : ☐ Male ☐Female ☐Neutral

		Can you explain to a peer :				
	I don't know what that is	**Not at all**	**A little**	**More or less**	**Pretty much**	**Perfectly**
The Structure of DNA	☐	☐	☐	☐	☐	☐
What is a Gene	☐	☐	☐	☐	☐	☐
What is a GMO	☐	☐	☐	☐	☐	☐
DNA replication	☐	☐	☐	☐	☐	☐
DNA amplification techniques	☐	☐	☐	☐	☐	☐
Fluorescence	☐	☐	☐	☐	☐	☐
What a wavelength is	☐	☐	☐	☐	☐	☐
Citizen Science	☐	☐	☐	☐	☐	☐

Please write some questions you have about Genetics/DNA/GMOs :

	I am interested in:						I am willing to invest time to know more about this:
	I don't know what that is	Not at all interested	A little interested	Neutral	Interested	Very interested	
Science	☐	☐	☐	☐	☐	☐	☐
Biologie	☐	☐	☐	☐	☐	☐	☐
Electronics	☐	☐	☐	☐	☐	☐	☐
Physics	☐	☐	☐	☐	☐	☐	☐
Citizen science	☐	☐	☐	☐	☐	☐	☐
Do It Yourself	☐	☐	☐	☐	☐	☐	☐
Open source	☐	☐	☐	☐	☐	☐	☐
Fablabs	☐	☐	☐	☐	☐	☐	☐

	Do you agree or disagree with the following:				
	Don't agree at all	**Disagree a little**	**Neutral**	**Agree a little**	**Completely agree**
I am against GMOs	☐	☐	☐	☐	☐
I'm willing to eat GMOs	☐	☐	☐	☐	☐
I'm willing to eat animal which ate GMO's	☐	☐	☐	☐	☐
Producing medicine/drugs from genetically modified bacteria	☐	☐	☐	☐	☐
Cleaning soil and water with genetically modified bacteria	☐	☐	☐	☐	☐
Curing genetic diseases by modifying the DNA of a patient	☐	☐	☐	☐	☐
I am ready to give my time to help advance science by helping scientists	☐	☐	☐	☐	☐
I see myself having a scientific career	☐	☐	☐	☐	☐

Are you excited to start the experiment?

Are you eager to learn more about genetics and GMOs?

After English:

GMO Workshop Questionnaire:

		Can you explain to a peer :				
	I don't know what that is	**Not at all**	**A little**	**More or less**	**Pretty much**	**Perfectly**
The Structure of DNA	☐	☐	☐	☐	☐	☐
What a Gene is	☐	☐	☐	☐	☐	☐
What a GMO is	☐	☐	☐	☐	☐	☐
DNA replication	☐	☐	☐	☐	☐	☐
DNA amplification techniques	☐	☐	☐	☐	☐	☐
Fluorescence	☐	☐	☐	☐	☐	☐
What a wavelength is	☐	☐	☐	☐	☐	☐
Citizen Science	☐	☐	☐	☐	☐	☐

Please write some questions you have about Genetics/DNA/GMOs :

	How would you describe the difficulty of understanding the following notions?				
	Very easy	Easy	Normal	Hard	Very hard
The Structure of DNA	☐	☐	☐	☐	☐
What a Gene is	☐	☐	☐	☐	☐
What transgenesis is	☐	☐	☐	☐	☐
DNA replication	☐	☐	☐	☐	☐
DNA amplification techniques	☐	☐	☐	☐	☐
What a Primer is	☐	☐	☐	☐	☐
Fluorescence	☐	☐	☐	☐	☐
Citizen Science	☐	☐	☐	☐	☐

	How much do you agree or disagree with the following statements?				
	Don't agree at all	Disagree a little	Neutral	Agree a little	Completely agree
I learned the importance of sharing knowledge	☐	☐	☐	☐	☐
I learned that I could take part in scientific research	☐	☐	☐	☐	☐
I found the experience hard to do	☐	☐	☐	☐	☐
I found the experience hard to understand	☐	☐	☐	☐	☐
I found the results hard to interpret	☐	☐	☐	☐	☐
I had a good time	☐	☐	☐	☐	☐
I was interested in what I did and learned	☐	☐	☐	☐	☐
This workshop made me want to do some research	☐	☐	☐	☐	☐
I can explain what is in each of the 8 tubes	☐	☐	☐	☐	☐
I can explain what a negative control is	☐	☐	☐	☐	☐
I can explain what a positive control is	☐	☐	☐	☐	☐
I am able to repeat this experiment with someone else	☐	☐	☐	☐	☐
I feel comfortable doing a scientific experiment	☐	☐	☐	☐	☐
I discovered that there are plenty of resources at my disposal which enable me to carry out my own actions	☐	☐	☐	☐	☐
I know what a Fablab is and what can be done there	☐	☐	☐	☐	☐

	I am interested in:						I am willing to invest time to know more about this:
	I don't know what that is	Not at all interested	A little interested	Neutral	Interested	Very interested	
Science	☐	☐	☐	☐	☐	☐	☐
Biologie	☐	☐	☐	☐	☐	☐	☐
Electronics	☐	☐	☐	☐	☐	☐	☐
Physics	☐	☐	☐	☐	☐	☐	☐
Citizen science	☐	☐	☐	☐	☐	☐	☐
Do It Yourself	☐	☐	☐	☐	☐	☐	☐
Open source	☐	☐	☐	☐	☐	☐	☐
Fablabs	☐	☐	☐	☐	☐	☐	☐

	As a result of this workshop, did you master the following skills?					
	Not at all	Not really	Neutral	I'm comfortable	I mastered perfectly	I already knew it
Using a micropipette	☐	☐	☐	☐	☐	☐
Conduction of a scientific experiment	☐	☐	☐	☐	☐	☐
Working with a group	☐	☐	☐	☐	☐	☐
Formulate ideas	☐	☐	☐	☐	☐	☐
Reflect on a socio-scientific subject with a critical eye	☐	☐	☐	☐	☐	☐
Generate and analyze data	☐	☐	☐	☐	☐	☐
Connect several disciplines / subjects together	☐	☐	☐	☐	☐	☐
Taking part in a citizen science project	☐	☐	☐	☐	☐	☐

	You agree or disagree with the following proposals				
	Don't agree at all	Disagree a little	Neutral	Agree a little	Completely agree
I am against GMOs	☐	☐	☐	☐	☐
I'm willing to eat GMOs	☐	☐	☐	☐	☐
I'm willing to eat animal which eat GMO's	☐	☐	☐	☐	☐
To produce medicine/drugs from genetically modified bacteria	☐	☐	☐	☐	☐
To clean soil and water with genetically modified bacteria	☐	☐	☐	☐	☐
To cure genetic diseases by modifying the DNA of a patient	☐	☐	☐	☐	☐
I am ready to give my time to help advance science by helping scientists	☐	☐	☐	☐	☐
I see myself having a scientific career	☐	☐	☐	☐	☐

PART BIOLOGY

What was the hardest part of the biological experiment?

What caused you problems in the biology part?

What has been the best part of the biological experiment for you?

What has been the worst part of the biological experiment for you?

Apart from GMOs, what would you like to detect with this method?

In what area/s do you think that genetic engineering interventions are useful and justified?

PHYSICAL AND ELECTRONIC PART

What was the hardest part of the Physics & Electronics part for you?

What was the best part of the Physics & Electronics part for you?

What was the worst part of the Physics & Electronics part for you?

GENERALLY

What did you prefer?

Do you have any comments?

French Before:

Questionnaire Atelier OGM - Début

Age :

Genre : ☐ Homme ☐Femme ☐Neutre

Niveau et filière de la classe :

		Pourriez-vous expliquer à votre entourage :				
	Je ne sais pas ce que c'est	**Pas du tout**	**Plutôt non**	**Plus ou moins**	**Plutôt oui**	**Parfaitement**
La structure de l'ADN	☐	☐	☐	☐	☐	☐
Ce qu'est un gène	☐	☐	☐	☐	☐	☐
Ce qu'est un OGM	☐	☐	☐	☐	☐	☐
La réplication de l'ADN	☐	☐	☐	☐	☐	☐
Les techniques d'amplification de l'ADN	☐	☐	☐	☐	☐	☐
La fluorescence	☐	☐	☐	☐	☐	☐
Ce qu'est une longueur d'onde	☐	☐	☐	☐	☐	☐
Les sciences citoyennes	☐	☐	☐	☐	☐	☐

Ecrivez quelques questions que vous vous poser sur la génétique / de l'ADN / des OGMs ? (Autant que vous voulez) :

		Je suis intéressé par :					Je suis prêt à consacrer du temps pour approfondir ce sujet :
	Je ne sais pas ce que c'est	Pas du tout Intéressé	Peu intéressé	Neutre	Intéressé	Très intéressé	
La science	☐	☐	☐	☐	☐	☐	☐
La biologie	☐	☐	☐	☐	☐	☐	☐
L'électronique	☐	☐	☐	☐	☐	☐	☐
La physique	☐	☐	☐	☐	☐	☐	☐
Les sciences citoyenne	☐	☐	☐	☐	☐	☐	☐
Le Do It Yourself	☐	☐	☐	☐	☐	☐	☐
L'open source	☐	☐	☐	☐	☐	☐	☐
Les Fablabs	☐	☐	☐	☐	☐	☐	☐

Savez-vous ce qu'est un Fablab ? ☐ Oui ☐ Non

Avez-vous déjà visité un Fablab ? ☐ Oui ☐ Non

Avez-vous déjà fait de la soudure ? ☐ Oui ☐ Non

Avez-vous déjà fait imprimer quelque chose en 3D ? ☐ Oui ☐ Non

Avez-vous déjà eu recours à du contenu open source ? ☐ Oui ☐ Non ☐ Je ne sais pas

Etes-vous impatient de commencer l'expérience ? ☐ Oui ☐ Non

Etes-vous impatient d'en apprendre plus sur la génétique et les OGMs ? ☐ Oui ☐ Non

Savez-vous ce qu'est la science citoyenne ? ☐ Oui ☐ Non

Etes-vous engagés dans le secteur associatif ? ☐ Oui ☐ Non

	Vous êtes en accord ou en désaccord avec les propositions suivantes				
	Pas du tout d'accord	Plutôt pas d'accord	Neutre	Un peu d'accord	Complément d'accord
Je suis contre les OGMs	☐	☐	☐	☐	☐
Je suis prêt à manger des OGMs	☐	☐	☐	☐	☐
Je suis prêt à manger des animaux qui ont mangé des OGMs	☐	☐	☐	☐	☐
Je suis d'accord pour faire produire des médicaments par des bactéries transformées génétiquement	☐	☐	☐	☐	☐
Je suis d'accord pour dépolluer le sol et l'eau grâce à des bactéries transformées	☐	☐	☐	☐	☐
Je suis d'accord pour que l'on guérisse les maladies d'origine génétique en modifiant l'ADN d'un patient	☐	☐	☐	☐	☐
Je suis prêt à m'engager et donner du temps pour aider à faire avancer la science en aidant des scientifiques	☐	☐	☐	☐	☐
J'envisage une carrière scientifique	☐	☐	☐	☐	☐

French After

Questionnaire Atelier OGM - Final

		Pourriez-vous expliquer à votre entourage :				
	Je ne sais pas ce que c'est	**Pas du tout**	**Plutôt non**	**Plus ou moins**	**Plutôt oui**	**Parfaitement**
La structure de l'ADN	☐	☐	☐	☐	☐	☐
Ce qu'est un gène	☐	☐	☐	☐	☐	☐
Ce qu'est un OGM	☐	☐	☐	☐	☐	☐
La réplication de l'ADN	☐	☐	☐	☐	☐	☐
Les techniques d'amplification de l'ADN	☐	☐	☐	☐	☐	☐
La fluorescence	☐	☐	☐	☐	☐	☐
Ce qu'est une longueur d'onde	☐	☐	☐	☐	☐	☐
Les sciences citoyennes	☐	☐	☐	☐	☐	☐

Ecrivez quelques questions que vous vous poser sur la génétique / de l'ADN / des OGMs ? (Autant que vous voulez) :

	Comment qualifieriez-vous la difficulté que vous avez eue à comprendre les notions suivantes ?				
	Très facile	Facile	Normal	Dur	Très dur
La structure de l'ADN	☐	☐	☐	☐	☐
Ce qu'est un gène	☐	☐	☐	☐	☐
Ce qu'est la transgenèse	☐	☐	☐	☐	☐
La réplication de l'ADN	☐	☐	☐	☐	☐
Les techniques d'amplification de l'ADN	☐	☐	☐	☐	☐
Ce qu'est un Primer ou Amorce	☐	☐	☐	☐	☐
La fluorescence	☐	☐	☐	☐	☐
Les sciences citoyennes	☐	☐	☐	☐	☐

	A quel point êtes-vous en accord ou en désaccord avec les propositions suivantes ?				
	Pas du tout d'accord	Plutôt pas d'accord	Neutre	Un peu d'accord	Complément d'accord
J'ai appris l'importance de mettre en commun le savoir	☐	☐	☐	☐	☐
J'ai appris que je pouvais prendre part à des recherches scientifiques	☐	☐	☐	☐	☐
J'ai trouvé l'expérience dure à réaliser	☐	☐	☐	☐	☐
J'ai trouvé l'expérience dure à comprendre	☐	☐	☐	☐	☐
J'ai trouvé les résultats durs à interpréter	☐	☐	☐	☐	☐
J'ai passé un bon moment	☐	☐	☐	☐	☐
J'ai été intéressé par ce que j'ai fait et appris	☐	☐	☐	☐	☐
Cet atelier m'a donné envie de faire de la recherche	☐	☐	☐	☐	☐
Je suis capable d'expliquer ce qui se trouve dans chacun des 8 tubes	☐	☐	☐	☐	☐
Je sais expliquer ce qu'est un contrôle négatif	☐	☐	☐	☐	☐
Je sais expliquer ce qu'est un contrôle positif	☐	☐	☐	☐	☐
Je suis capable de refaire cette expérience avec quelqu'un d'autre	☐	☐	☐	☐	☐
Je me sens à l'aise avec la réalisation d'une expérience scientifique	☐	☐	☐	☐	☐
J'ai découvert qu'il y avait plein de ressources à ma disposition pour mener mes propres actions	☐	☐	☐	☐	☐
Je sais ce qu'est un Fablab et ce que l'on peut y faire	☐	☐	☐	☐	☐

		Je suis intéressé par :					Je suis prêt à consacrer du temps pour approfondir ce sujet :
	Je ne sais pas ce que c'est	Pas du tout Intéressé	Peu intéressé	Neutre	Intéressé	Très intéressé	
La science	☐	☐	☐	☐	☐	☐	☐
La biologie	☐	☐	☐	☐	☐	☐	☐
L'électronique	☐	☐	☐	☐	☐	☐	☐
La physique	☐	☐	☐	☐	☐	☐	☐
Les sciences citoyenne	☐	☐	☐	☐	☐	☐	☐
Le Do It Yourself	☐	☐	☐	☐	☐	☐	☐
L'open source	☐	☐	☐	☐	☐	☐	☐
Les Fablabs	☐	☐	☐	☐	☐	☐	☐

	A la suite de cet atelier, maitrisez-vous les compétences suivantes ?					
	Je ne maîtrise pas du tout	Je ne suis pas à l'aise	Neutre	Je suis à l'aise	Je maîtrise Parfaitement	Je maitrisais déjà avant
Utilisation d'une micropipette	☐	☐	☐	☐	☐	☐
Réalisation d'une expérience scientifique	☐	☐	☐	☐	☐	☐
Travailler en groupe	☐	☐	☐	☐	☐	☐
Formuler des idées	☐	☐	☐	☐	☐	☐
Réfléchir un sujet scientifique avec un œil critique	☐	☐	☐	☐	☐	☐
Générer et analyser des données	☐	☐	☐	☐	☐	☐
Connecter plusieurs disciplines / matières entre elles	☐	☐	☐	☐	☐	☐
Prendre part à un projet de science citoyenne	☐	☐	☐	☐	☐	☐

	Vous êtes en accord ou en désaccord avec les propositions suivantes				
	Pas du tout d'accord	Plutôt pas d'accord	Neutre	Un peu d'accord	Complément d'accord
Je suis contre les OGMs	☐	☐	☐	☐	☐
Je suis prêt à manger des OGMs	☐	☐	☐	☐	☐
Je suis prêt à manger des animaux qui ont mangé des OGMs	☐	☐	☐	☐	☐
Je suis d'accord pour faire produire des médicaments par des bactéries transformées génétiquement	☐	☐	☐	☐	☐
Je suis d'accord pour dépolluer le sol et l'eau grâce à des bactéries transformées	☐	☐	☐	☐	☐
Je suis d'accord pour que l'on guérisse les maladies d'origine génétique en modifiant l'ADN d'un patient	☐	☐	☐	☐	☐
Je suis prêt à m'engager et donner du temps pour aider à faire avancer la science en aidant des scientifiques	☐	☐	☐	☐	☐
J'envisage une carrière scientifique	☐	☐	☐	☐	☐

PARTIE BIOLOGIE

Quelle a été pour vous la partie la plus dure de l'expérience biologique ?

Qu'est-ce qui vous a posé problèmes dans la partie biologie ?

Quelle a été pour vous la meilleure partie de l'expérience biologique ?

Quelle a été pour vous la pire partie de l'expérience biologique ?

Mise à part les OGMs, qu'aimeriez-vous détecter grâce à cette expérience ?

Dans quel domaine pensez-vous que l'intervention du génie génétique soit utile et justifiée ? Merci de séparer vos réponses par des virgules.

PARTIE PHYSIQUE ET ELECTRONIQUE

Quelle a été pour vous la partie la plus dure de la partie Physique & Electronique ?

Quelle a été pour vous la meilleure partie de la partie Physique & Electronique ?

Quelle a été pour vous la pire partie de la partie Physique & Electronique ?

DE MANIERE GENERALE

Qu'avez-vous préféré ?

Avez-vous des remarques ?

Digital versions of the questionnaires in English available here:

https://bit.ly/323xnqU

https://bit.ly/2FOMLhr

And in spanish here:

https://forms.gle/cfkYYVNA1SzRKsfd8

https://forms.gle/wsENrZFVXN2njksP8

S SVT					
	Première			Terminale	
Thématique	**Connaissances**	**Compétences**	Thématique	**Connaissances**	**Compétences**
Thème 1 - A Expression, stabilité et variation du patrimoine génétique	En absence d'erreur, ce phénomène préserve, par copie conforme, la séquence des nucléotides. Ainsi, les deux cellules filles provenant par mitose d'une cellule mère possèdent la même information génétique. La séquence des nucléotides d'une molécule d'ADN représente une information. Le code génétique est le système de correspondance mis en jeu lors de la traduction de cette information.	Mettre en oeuvre une méthode (démarche historique) et/ou une utilisation de logiciels et/ou une pratique documentaire permettant de comprendre le mécanisme de réplication semi conservative.	**Thème 1 - La Terre dans l'Univers, la vie, l'évolution du vivant Thème 1-A Génétique et évolution**	Bilan de la classe de Première, le Kit peut être l'occasion de faire un rappel général sur les bases de génétique.	
			Thème 2-B La plante domestiquée	La sélection exercée par l'Homme sur les plantes cultivées a souvent retenu (volontairement ou empiriquement) des caractéristiques génétiques différentes de celles qui sont favorables pour les plantes sauvages. Une même espèce cultivée comporte souvent plusieurs variétés sélectionnées selon des critères différents ; c'est une forme de biodiversité. Les techniques de croisement permettent d'obtenir de nouvelles plantes qui n'existaient pas dans la nature (nouvelles variétés, hybrides, etc.). Les techniques du génie génétique permettent d'agir directement sur le génome des plantes cultivées	Comparer une plante cultivée et son ancêtre naturel supposé. Recenser, extraire et exploiter des informations afin de comprendre les caractéristiques de la modification génétique d'une plante.
Thème 2 - B Nourrir l'humanité	L'agriculture repose sur la constitution d' agrosystèmes gérés dans le but de fournir des produits (dont les aliments) nécessaires à l'humanité.	Faire preuve d'esprit critique en étudiant la conduite d'une culture quant à son impact sur l' environnement.			
Thème 3 - B Variation génétique et santé		établir le lien entre le phénotype macroscopique et le génotype			
Objectifs transversaux	Pratiquer une démarche scientifique (observer, questionner, formuler une hypothèse, expérimenter, raisonner avec rigueur, modéliser). Recenser, extraire et organiser des informations. Manipuler et expérimenter Percevoir le lien entre sciences et techniques. Manifester sens de l'observation, curiosité, esprit critique. Être conscient de sa responsabilité face à l'environnement, la santé, le monde vivant Être capable d'attitude critique face aux ressources documentaires. Manifester de l'intérêt pour la vie publique et les grands enjeux de la société.				

S Enseignement moral et civile				
	Première		Terminale	
Connaissances	Exercer sa citoyenneté dans la République française et l'Union européenne	S'engager : la notion de militantisme ; les grandes formes d'engagement politique, syndical, associatif.	Biologie, éthique, société et environnement	La notion de bioéthique. Les problèmes bioéthiques contemporains (la recherche génétique, les OGM, les thérapies géniques...). Le rôle du Haut Conseil des biotechnologies.
		L'idée de citoyenneté européenne.		Les évolutions de l'éthique médicale. La question du consentement du patient. Le rôle du Comité consultatif national d'éthique (CCNE).
	Les enjeux moraux et civiques de la société de l'information	Spécificité et rôle des différents médias et éléments de méthode permettant la compréhension critique des informations dont ils sont porteurs et des réactions qu'ils suscitent (commentaires interactifs, blogs, tweets...).		La responsabilité environnementale. L'interdépendance humanité-nature. Le principe de précaution : sa réalité juridique, ses applications et ses limites.
Compétences	Identifier et expliciter les valeurs éthiques et les principes civiques en jeu. Mobiliser les connaissances exigibles. Développer l'expression personnelle, l'argumentation et le sens critique. S'impliquer dans le travail en équipe.			

Philosophie		
	Thématique	**Compétences**
Au programme	L'art	Générer des idées
	Le vivant	Débattre
	La vérité	Sens Critique
	La société et l'État	
Hors programme	La bioéthique	

STL

Chimie, science du vivant

Première			Terminale		
Thématique	**Connaissances**	**Compétences**	Thématique	**Connaissances**	**Compétences**
4.1 Les propriétés informatives de l'ADN sont liées à sa structure	Un nucléotide de l'ADN est constitué d'une base azotée, d'un désoxyribose, et d'un groupement phosphate. Structure primaire de l'ADN, la séquence orientée des nucléotides constitue le support de l'information. Les interactions hydrogène entre les bases azotées permettent l'association de deux brins complémentaires en double hélice.	- déduire l'importance de l'ADN dans l'acquisition de phénotypes nouveaux : notion de principe transformant. Exploiter des ressources documentaires pour : - identifier les constituants de l'ADN ; - établir la séquence complémentaire d'une séquence désoxyribonucléotidique donnée. **exercer les élèves à conduire une dialectique entre les faits et les idées**	**4.2 Le phénotype d' un individu est lié à l' expression de son génotype**	Le phénotype est constitué par les caractères visibles d'un individu. Le génotype est l'ensemble des allèles d'un individu.	- proposer une définition d'un gène ;
			4.3 La séquence codante d'un gène permet l'expression d'un caractère via la synthèse d'une protéine	L'ADN contient des unités d'information appelées gènes La séquence d'ADN du brin codant d'un gène est transcrite en une séquence d'ARN : c'est la transcription Dans le cas de l'ARN messager, la séquence codante est traduite en une séquence d'acides aminés selon le code génétique universel : c' est la traduction.	- construire un schéma simplifié : . localisant la transcription et la traduction dans la cellule, . de la phase d'élongation de la transcription, . de la phase d'élongation de la traduction.
			4.4 L'information génétique est conservée par réplication de l'ADN	La réplication de l'ADN est semi-conservative chez les procaryotes comme chez les eucaryotes.	Exploiter des ressources documentaires pour : - mettre en relation la structure double brin de la molécule d'ADN avec la réplication ; - analyser une expérience montrant le caractère semiconservatif de la réplication
			4.6 L'ADN est un objet des biotechnologies	Le patrimoine génétique d'un organisme peut être modifié afin de conférer à cet organisme de nouvelles potentialités. Des recherches en thérapie génique explorent des pistes pour soigner les maladies génétiques.	Exploiter des ressources documentaires pour : - identifier des domaines de production faisant intervenir des organismes génétiquement modifiés ; - identifier la potentialité recherchée par une modification génétique et les éventuels risques associés. Exploiter des ressources documentaires pour : - expliquer la stratégie mise en œuvre dans une thérapie génique ; - distinguer, pour une thérapie génique donnée, ce qui est en cours de réalisation de ce qui est prospectif.

STL

Enseignement de biotechnologie

Première			Terminale		
Thématique	**Connaissances**	**Compétences**	Thématique	**Connaissances**	**Compétences**
			Biotechnologies : éthique, impact économique et démarche technologique	- Impact économique, valeur ajoutée d'un produit Regard critique sur la médiatisation des biotechnologies Éthique (en lien avec l'enseignement de philosophie) L'objectif est de développer chez les élèves un sens critique et de dépasser la réflexion purement scientifique et technologique.	Se questionner sur les conséquences des applications et des procédés des biotechnologies, en lien avec l'actualité. - Développer sa responsabilité de citoyen face aux questions de la bioéthique. - Conduire une recherche documentaire. - Travailler en équipe. À partir d'articles scientifiques ou non, choisis dans l' actualité, les élèves seront sensibilisés à l' impact des biotechnologies à l'aide d'un questionnement judicieux. - Identifier les étapes critiques d'une méthode pour prévenir les risques. - Respecter un protocole, une méthode normalisée utiliser un contrôle (étalon de travail) pour valider la qualité d'une manipulation critiquer un résultat
			Initiation à la biologie moléculaire et au génie génétique	Code génétique et traduction de séquence nucléotidique - Polymérisation des acides nucléiques et amplification en chaîne par polymérisation (ACP ou PCR). - Vecteurs d'amplification et d'expression : . marqueur de sélection, . origine de réplication, . promoteur, . site de clonage multiple, . gène rapporteur, . gène d'intérêt Méthodes d'identification moléculaire : - Clonage et OGM. Caractère génétique d'une maladie héréditaire et thérapie génique.	Identifier les points critiques d'un pipetage de micro volumes S'assurer de la qualité du matériel de prélèvement - Conditionner, étiqueter et conserver (congélation ou non) les échantillons, les réactifs, etc - Exploiter des documents pour appréhender les limites, l'intérêt et le contexte d'utilisation de différentes méthodes

STL et S				
	Enseignement moral et civile			
	Première		Terminale	
Connaissances	Exercer sa citoyenneté dans la République française et l'Union européenne	S'engager : la notion de militantisme ; les grandes formes d'engagement politique, syndical, associatif.	Biologie, éthique, société et environnement	La notion de bioéthique. Les problèmes bioéthiques contemporains (la recherche génétique, les OGM, les thérapies géniques...). Le rôle du Haut Conseil des biotechnologies.
		L'idée de citoyenneté européenne.		Les évolutions de l'éthique médicale. La question du consentement du patient. Le rôle du Comité consultatif national d'éthique (CCNE).
	Les enjeux moraux et civiques de la société de l'information	Spécificité et rôle des différents médias et éléments de méthode permettant la compréhension critique des informations dont ils sont porteurs et des réactions qu'ils suscitent (commentaires interactifs, blogs, tweets...).		La responsabilité environnementale. L'interdépendance humanité-nature. Le principe de précaution : sa réalité juridique, ses applications et ses limites.
Compétences	Identifier et expliciter les valeurs éthiques et les principes civiques en jeu.			
	Mobiliser les connaissances exigibles.			
	Développer l'expression personnelle, l'argumentation et le sens critique.			
	S'impliquer dans le travail en équipe.			

H. Some representative workshop photos



High School Workshops



MENNECY, FRANCE



#citizenscience



#education

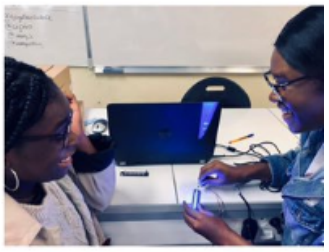




High School Workshops



LES ULIS, FRANCE



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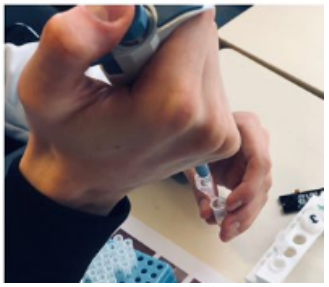
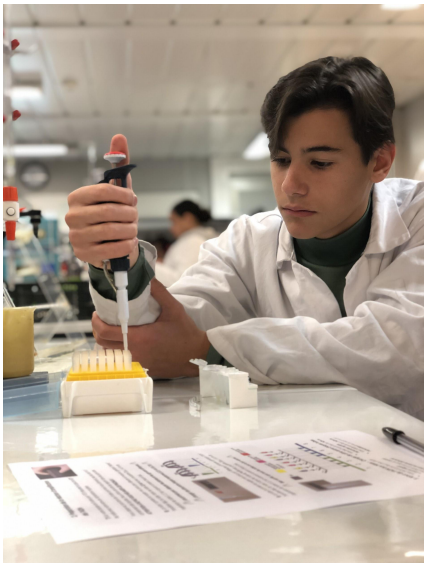
#DoItYourself











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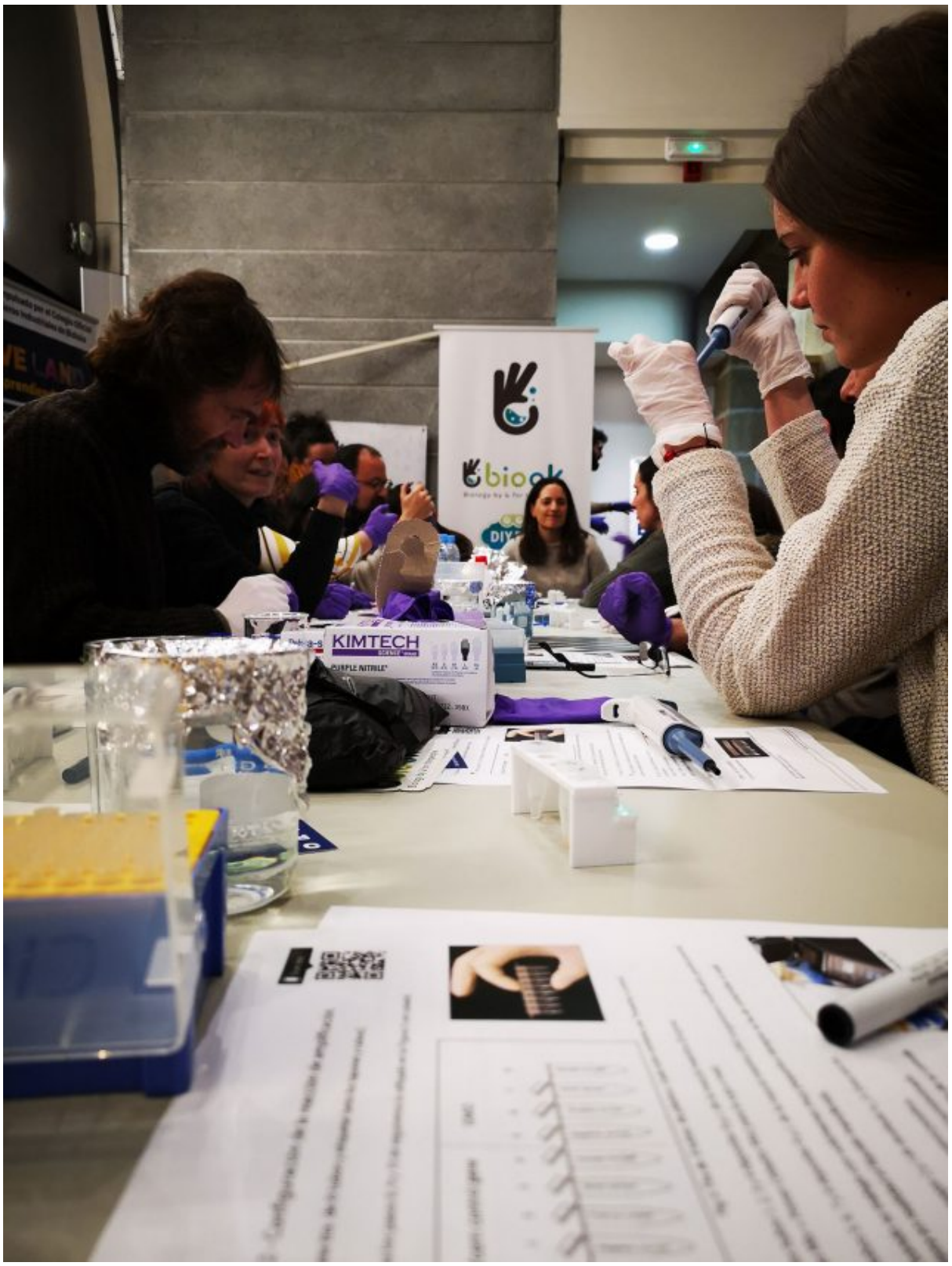
KIMTECH
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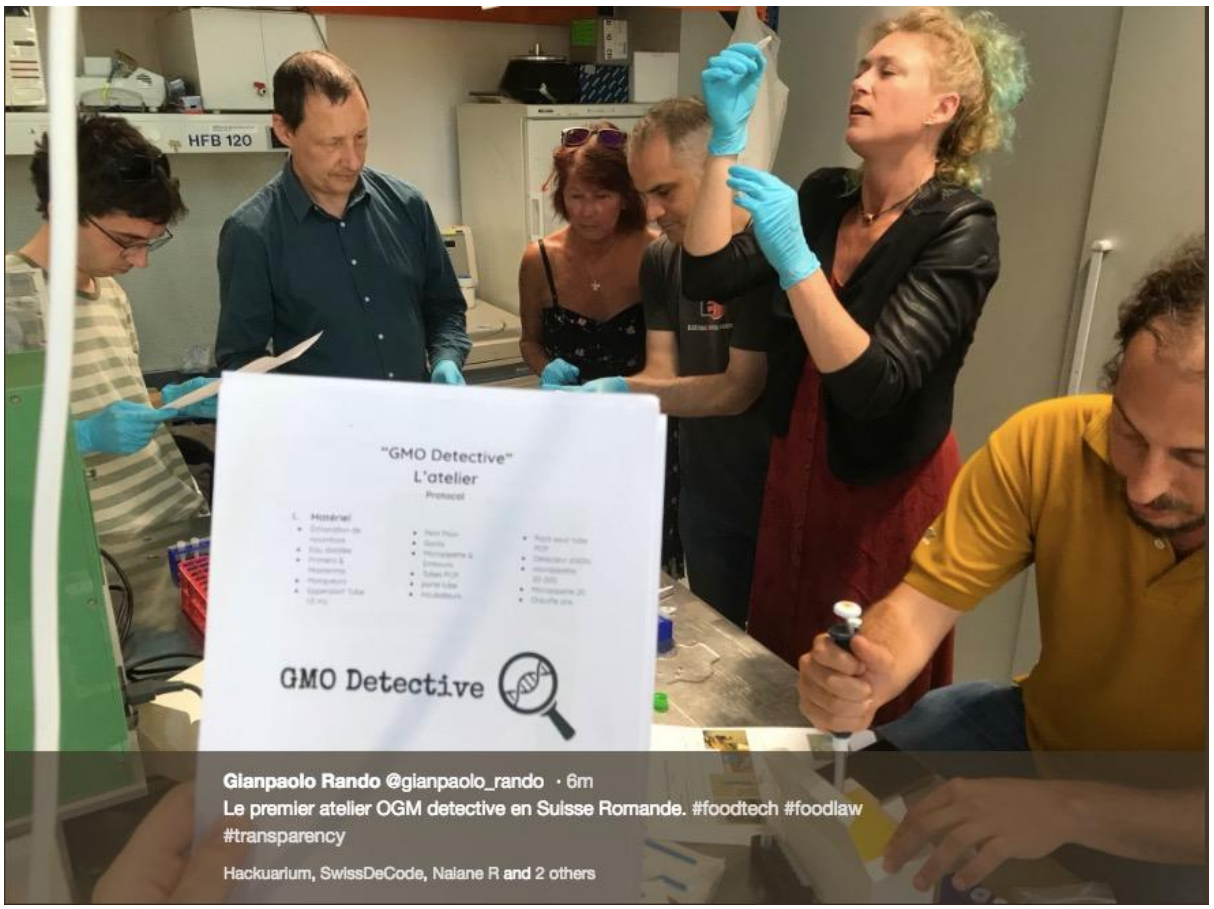

https://www.youtube.com/watch?v=rfHsYSdNRbE

https://www.youtube.com/watch?v=671vCuxIQ9o