“*TetI-Box*” an accessible synthetic biology toolbox

Introduction

Developing science in LATAM can often be expensive and hard to access, limiting scientific progress and development given the low-budget laboratories are given.
Particularly in Mexico, access to science at any level of education is not limited only by financial reasons. Other factors such as import regulations applicable to the reagents and equipment importation also have a considerable impact. These circumstances cause many institutions to find themselves without any tools or resources to share this knowledge with their students. That is why we decided to create TetI-box, a project aimed to create the opportunity for students to approach science at the early stages of education.

TetI-Box is a synthetic biology toolbox, a compendium of essential tools in the form of DIY reagents that let students have a safe, affordable, and simple approach to biotechnology, molecular biology, and synthetic biology through a series of lab protocols for DNA extraction, enzymatic digestion, and ligation, cell transformation, biobrick obtention, and PCR.
To allow the production of these elements, we designed a low-cost reactant-producing genetic circuit with the following biological parts:

BBa_I0500(AraC CDS+AraBAD Promoter)
BBa_B0015(Double terminator)
BBa_B0034 (Elowitz RBS)
BBa_I746350 (Self-regulated CDS)
BBa_I746360 (BBa_I746350 promoter)

Our genetic circuit design consists of a positive feedback loop induced by a small amount of arabinose, allowing users to obtain the reagents in a safe, constant, and controlled way.
The circuit allows the insertion of different CDS for protein expression, we designed 13 exogenous proteins optimized via codon edition and engineered with prefixes and suffixes to allow insertion into the circuit. .
We went further with the DNA Taq polymerase by modifying its native sequence so that it acquires retrotranscriptase activity as well as higher processivity and fidelity.
This genetic circuit is inserted in a vector we designed by modifying the existing part *BBa_K823026* making it simpler and more compatible with the chosen chassis, E. coli BL21.

To create a better design, we developed two models: An ODE mathematical model that allowed us to study equilibrium states and system stability besides the circuit properties, for example, high concentration of the protein of interest produced by low inductor inputs. This model helped us obtain a mathematical expression capable of describing the quantity of produced reagent.
The second model was a cellular automata model, which offers a new perspective, given its differences from the ODE model. This one makes a stochastic analysis and describes each cell individually instead of the whole cellular population.

We analyzed all these circuits, vectors, and biobrick on pDRAW32 and SnapGene software then protein structures were predicted using ROBETTA and EXPASY's SwissModel. Specifically for the modified Taq polymerase we conducted a docking analysis to ensure the protein's activity against its ligand, we also conducted the same analysis on the native Taq polymerase and the result comparison showed that our modified protein had a better docking score and ligand rmsd score (A).

To ensure we are designing a safe, ethical, and consistent project, we've analyzed both the problem and our solution from a technical, bioethical, and social perspective. We achieved this through several data collection methods, such as direct consults with stakeholders, interviews, surveys, and focus groups. We also implemented experimental design techniques to elaborate a strategy to test the resources created. The results obtained were used to quantitatively and qualitatively measure the project's impact and evaluate the fulfillment of our team's objectives.
We added a killswitch to our expression vector that works in two ways: as a programmed cell death, where the user can induce cell death by adding lactose and also as a safety net mechanism, where in case of accidental spill of media in sewage the circuit will be activated as lactose is one of the most common sugars found in residual water.

The team also identified vulnerable groups that face even more social and economic barriers, without mentioning discrimination and segregation practices from their fellow citizens (kids, disabled people, and native communities). These issues together make becoming a member of the scientific community an impossible task. Overcoming these barriers can give these vulnerable groups an opportunity. A deep analysis of their experiences and situations helps our project to reach more people, guiding us to make synthetic biology knowledge accessible.

Seeking that science and technology are available to everyone, we set ourselves the objective of making biotechnology known to vulnerable groups by overcoming the difficulty of access to the small communities to which they belong.

With the help of various stakeholders, we generated our educational book "Ollibro" which seeks to introduce science in their communities and encourage creativity among children or anyone interested in learning. Promoting throughout activities and experiments the development of knowledge helps them understand how the elements around them operate and how they could provide even more wisdom for their everyday life. (For more information about Ollibro's development, go to Diversity and Inclusion appendix).

Considering both the project scope and the proposed experimental practices for the students, we implemented a risk assessment system that aims to create a project designed with safety in mind.
The system was developed according to WHO guidelines and drugs regulation authorities policies from different regions. Adapting the process to the design stage and considering the presented differences due to the nature of the project.
The first step consisted of identifying the risks that could arise in the implementation of Tetl-Box, for the users, the environment and other organisms. The risks were categorized according to the implementation stage in which they could occur:

- Workspace and reagents preparation.

- Use of Tetl-Box for the proposed experimental practices.
- Waste disposal, cleaning, and disinfection of the workspace.
- End-use of the product obtained using Tet-Box reagents.

Once categorized, each risk was analyzed to find its root cause and proposed the actions required to prevent, reduce or eradicate it. The results of this analysis were registered in the CAPA report (The general form is annexed to this document). The risks were described in the report, together with the tool used for the root cause analysis (Ishikawa diagram, cause-and-effect tree, etc.). It also includes the preventive or corrective action plan with target implementation dates.

As a result of this analysis, modifications were proposed to prevent, reduce or eliminate 42 identified risks. Among these preventive and corrective actions, two of them stand out:

- The integration of a caspases system into the genetic circuit.
- The Biosafety Guidelines and Bioethical Principles, a resource for the users of Tetl-Box.

The risk management system is not meant to be used as a one-time activity but an ongoing process. The methodology established will be used in the upcoming development steps.

This project can not become a reality without evaluating its feasibility and the possibility of benefiting the stakeholders. To accomplish this, the team received training in topics like entrepreneurship and personal finance by participating in online courses and programs like iGEM EPIC. With this training, the team developed a market, cost, and product competition analysis. We also conducted interviews with future users and clients; this data led the team to create the entrepreneurship vision to advance the project.

In essence, everything we developed intends to have an integrated approach that better contributes to solving the increasing inaccessibility of science, taking into account the many layers of this problem.

Materials and methods

Vector

Vector optimization

We chose to redesign the plasmid developed by the iGEM12_LMU-Munich team (*BBa_K823026*) (http://parts.igem.org/Part:BBa_K823026). This original part is an expression vector for *Bacillus subtilis*, which is replicative in both *Escherichia coli* and *B. subtilis*. It has resistance to ampicillin for cloning in E. coli and resistance to kanamycin for selection in *B. subtilis*. The multiple cloning site is downstream of a Pspac promoter, which is IPTG-inducible.

The selection of this plasmid was based on three principles: it is a simple backbone that has resistance to more than one antibiotic which is compatible with *E. coli* BL21 strain, both antibiotics are accessible and commonly used in synthetic biology and other fields and, last but not least, this part has shown that it works. The certainty of working with this plasmid is

justified in the results obtained by the Münich team (2012) who proposed this plasmid with both resistance genes (Kan and Amp) and later supported by the Toulouse team (2016), when reporting the functioning of the same, having only left the gene for resistance to Kanamycin; therefore, this evidence made us infer it's highly possible that the proposed plasmids are functional.

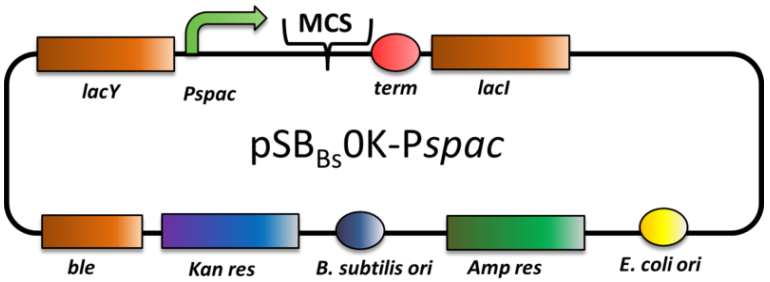


Figure 1. Original *BBa_K823026*, presented by the iGEM 2012 Münich team.

However, the team reported that the plasmid did not work as expected, as the promoter turned out to be strongly constitutive rather than induced by IPTG.

The general idea was to take this plasmid and remove all the genes that were unnecessary for our design (LacI, LacY and B. subtilis ori) in order to reduce its genetic load and increase its transformation efficiency, basing its deletion on the selected induction method, which is not IPTG-inducible (making the presence of these genes unnecessary). In the case of the origin of replication of *B. subtilis*, it is considered unnecessary, when working in an *E. coli* system. Subsequently, the construct resulting from the deletion of the three mentioned genes was used to develop two different plasmids. The first one includes the resistance genes to Ampicillin and Bleomycin, the second one, the resistance genes that code for genes Kanamycin and Bleomycin.
The advantage of having these two plasmids is that it presents the option to choose depending on the antibiotic preference and availability. Individually, the presence of two resistance genes in the same plasmid serves to axeny the culture.
In order to ensure that our constructs will work when tested in the lab, we searched for references that could be extrapolated to our work. For example, in 2016 the Toulouse team used the same part leaving just the resistance gene for Kanamycin and reported that it worked. Based on this information we can suppose that the similar modification that we made will work as well.
For the sequences we used in the plasmid, we took them from reliable databases such as NCBI, UniProt, BRENDA, Swiss-Model and so on. This allowed us to be sure that they worked fine and did what we intended them to do.

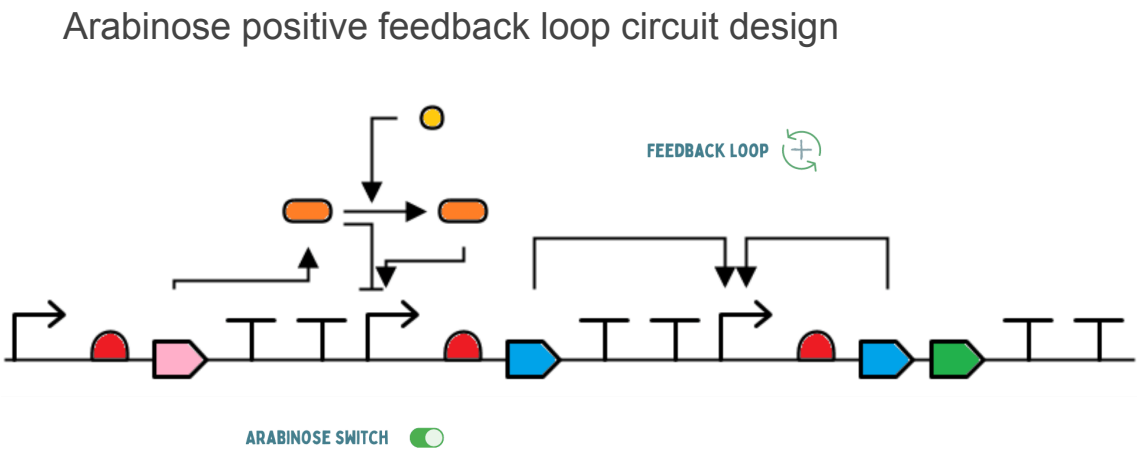


Figure 2. Arabinose positive feedback loop circuit

The low-cost arabinose induced genetic circuit is a positive feedback loop activatable with this sugar that can't be metabolized by Escherichia coli in the presence of glucose.
The first part of the circuit produces constitutively the AraC molecule (pink, *BBa_I0500*), which acts as a basal production repressor while arabinose is not present. Once arabinose (yellow) is added to the system, AraC changes from a repressor to a transcription inductor (orange) turning the circuit ON. With the circuit ON, the first activator protein starts transcribing (blue, *BBa_I746350*). This starts the positive feedback loop in which the production of the target protein (Target protein) continues even after initial inductor (yellow) depletion.

Table 1. Designed 18 exogenous protein-coding parts that can be easily inserted into the circuit

Taq/TBD polymerase	Bst DNA polymerase	Pfu DNA polymerase
Ligase	Protease	DNAse I
RNAse I	Xba I exonuclease	EcoRI exonuclease
Pst I exonuclease	NotI exonuclease	SpeI exonuclease
Green fluorescent protein (GFP)	Red fluorescent protein (mRFP)	aeBLUE chromoprotein
Invertase	Peroxidase	Acetyl transferase

Further information of these parts can be found on the appendices of this document.
Protocols:

- Experimental Protocol for standard PCR (Polymerase Chain-Reaction).
- Experimental Protocol for electrophoresis in agarose gel.
- Experimental Protocol for enzyme digestion and ligation.
- Experimental Protocol for plasmid extraction (Miniprep)
- Experimental Protocol for Transformation.

Killswitch

We also added a caspase circuit within the plasmid. We consider it as a biocontainment switch that helps us to increase the biosecurity measures of our modified bacterias. We made this circuit based on different articles which gave us information about how caspase of other organisms can induce apoptosis and why we cannot use the caspase of E.coli . The mechanism and further information is explained in the Policy, biosafety and/or biosecurity part.

Genetic circuit modelling

Mathematical model

With the simplification of the differential equations that will describe the system dynamics as a goal, some hypotheses will be assumed. The approach here used to propose the differential and chemical equations is explained in Marchisio, 2018. Here, the promoter is assumed as a representation of the whole transcriptional unit. Transcription and translation are treated as a single step, this implies that mRNA and ribosomes are not present in this model. Given the RNA polymerase concentration is usually a lot higher than that of the plasmid containing the circuit, it is assumed its concentration does not change based on the interactions with the circuit. Thus, RNA polymerase does not appear in this model. Variations in concentration by cell division are also not accounted for.
Let be the circuit shown in figure 2, assuming enough time has passed (time τ1), AraC protein is producing constitutively and has reached an equilibrium concentration and thus it is constant. It is also assumed enough time has passed (τ2) for araC to diffuse into the medium and join to the P1 promoter acting as a repressor. Once time τ1 + τ2 has passed, it is assumed arabinose has been added. Knowing araC protein maintains itself attached to the promoter forming a complex in presence or not of arabinose Martin et al., 1986, gives us reasons to consider the concentration of P1 promoter not attached to araC as negligible. According to Schleif, 2000 arabinose can associate to araC even if it is already forming a complex with the promoter, meaning, it is not necessary for araC to be free to associate with arabinose. These ideas are resumed in the following chemical equation:(In this work the following notation will be considered, let a transcription regulatory protein G, then G+ represents its conformation in which it can activate, G− represents its conformation in which it can repress, now, let P be a regulatable promoter, then P+ represents the activated promoter, P− represents the inhibited promoter and P represents the promoter in its basal state.)

$$(\mathrm{AraC}^- - \mathrm{P}_1) + n\,\mathrm{Ara} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{P}_1{}^+ \qquad \text{(II-A.1)}$$

Where ($AraC^-$−P_1) is the promoter-repressor complex, Ara is arabinose and P_1^+ is the promoter-activator complex (an activated promoter). This promoter can attract the RNA polymerase, initiating transcription and then translation of activator A. This promoter is also an inexhaustible reagent:

$$\mathrm{P_1}^{+} \xrightarrow{k_3} \mathrm{A} + \mathrm{P_1}^{+} \qquad \text{(II-A.2)}$$

The activator protein A can attach itself to promoter P_2, activating it.

$$\mathrm{A} + \mathrm{P_2} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} [P_2^{+}] \qquad \text{(II-A.3)}$$

now, this activated promoter [P_2^{+}] can attract RNA polymerase, start transcription and then translation of the activator A and the protein of interest, in this case GFP and as in the previous case, it is an inexhaustible reagent

$$[P_2^{+}] \xrightarrow{k_5} \mathrm{A} + \mathrm{GFP} + [P_2^{+}] \qquad \text{(II-A.4)}$$

Basal expression is a process to keep in mind in this model and it is considered in the following equation.

$$\mathrm{P_2} \xrightarrow{k_{lk}} \mathrm{A} + \mathrm{GFP} + \mathrm{P_2} \qquad \text{(II-A.5)}$$

Lastly, activator A and target protein GFP degradation were also considered due to physical and biological causes.

$$\mathrm{A} \xrightarrow{k_{d,A}} \qquad \text{(II-A.6)}$$

$$\mathrm{GFP} \xrightarrow{k_{d,G}} \qquad \text{(II-A.7)}$$

Differential Equations

Once the chemical equations have been presented a differential equation model based on the law of mass action can be constructed. It can be observed that k5 >> klk(see equations 2.1.4 y 2.1.5), meaning basal transcription rate is much lower than activator mediated transcription. This is valid for positively regulated promoters. Now, be [P1,T] the total concentration of promoter 1, and with the previous arguments:

$$[P_{1,T}] = \left[AraC^{-} - P\right] + \left[P_1^{+}\right] \qquad \text{(II-B.1)}$$

Now [P1+] dynamics Is given by(The constants used are just reaction rate constants obtained in the previous reactions.)

$$\frac{d[P_1^+]}{dt} = k_1[Ara]^n[AraC^- - P_1] - k_{-1}[P_1^+] \qquad \text{(II-B.2)}$$

Some time after adding arabinose is reasonable to think that P_1^+ will arrive at a constant equilibrium concentration, i.e., $\frac{d[P_1^+]}{dt}$ = 0.Using equation 2.2.1 and rearranging it the following equality is obtained:

$$[P_1^+] = \frac{[Ara]^n [P_{1,T}]}{H^n + [Ara]^n} \qquad \text{(II-B.3)}$$

Where

$$H^n = \frac{k_{-1}}{k_1}$$

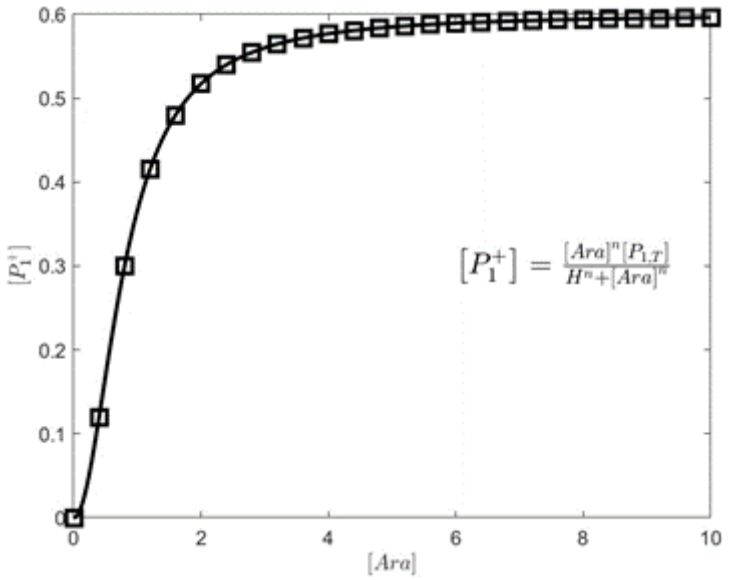


Figure 3: The [P_1^+] - [Ara] dependency is described via a Hill function. The following parameters were chosen 0 ≤ [*Ara*] ≤ 10;*n* = 2;[$P_{1,T}$] = 0.6;H^n = 0.8

H being the Hill constant and *n* the Hill coefficient. Through simplifications and assumed hypotheses a Hill function capable of modelling P_1^+ concentration based on Ara concentration was obtained. This relation is the key to the one between GFP concentration and Ara. In figure 3 a graphical representation can be seen, the parameters were chosen arbitrarily. Now the complete dynamics of the circuit can be modelled. The

dynamic of the Promoter 2 attached to activator protein $\left[P_2^+\right]$, activator protein [*A*] and target protein [*GFP*] are of special interest

$$\frac{d\left[P_2^+\right]}{dt} = k_4\left[A\right]\left[P_2\right] - k_{-4}\left[P_2^+\right] \qquad \text{(II-B.4)}$$

$$\begin{aligned}\frac{d\left[A\right]}{dt} = {} & k_3\left[P_1^+\right] + k_{-4}\left[P_2^+\right] \\ & + k_5\left[P_2^+\right] + k_{lk}\left[P_2\right] \\ & - k_4\left[A\right]\left[P_2\right] - k_{d,A}\left[A\right]\end{aligned} \qquad \text{(II-B.5)}$$

$$\frac{d\left[GFP\right]}{dt} = k_5\left[P_2^+\right] + k_{lk}\left[P_2\right] - k_{d,G}\left[GFP\right] \qquad \text{(II-B.6)}$$

Equations 2.2.4 y 2.2.5 are coupled and thus their analysis is fundamental to understand the circuit dynamics. The total concentration of promoter 2 [$P_{2,T}$] is constant and is given by

$$\left[P_{2,T}\right] = \left[P_2\right] + \left[P_2^+\right] \qquad \text{(II-B.7)}$$

Substituting equations 2.2.4 y 2.2.5 and rearranging

$$\frac{d\left[P_2^+\right]}{dt} = k_4\left[A\right]\left[P_{2,T}\right] - k_4\left[A\right]\left[P_2^+\right] - k_{-4}\left[P_2^+\right] \qquad \text{(II-B.8)}$$

$$\begin{aligned}\frac{d\left[A\right]}{dt} = {} & k_3\left[P_1^+\right] + k_{lk}\left[P_{2,T}\right] + \left[P_2^+\right]\left(k_{-4} + k_5 - k_{lk}\right) \\ & - \left[A\right]\left(k_4\left[P_{2,T}\right] + k_{d,A}\right) + k_4\left[A\right]\left[P_2^+\right]\end{aligned} \qquad \text{(II-B.9)}$$

In dimensionless form (The equation system non-dimensionalization is a long and technical process, and therefore has been omitted from this document.)

$$\frac{dx}{d\tau} = ay - xy - bx \qquad \text{(II-B.10)}$$

$$\frac{dy}{d\tau} = c + dx + xy - y \qquad \text{(II-B.11)}$$

Where (Variable and non-dimensional constant's meaning can be deducted from its definition:

- x is the non-dimensional concentration of activated promoter 2.
- y is the non-dimensional activator concentration.
- τ is the non-dimensional time.
- a is a measure of the activator protein kinetic stability.
- b is a measure of the dissociation force between the activator protein and its promoter.
- c is a measure of arabinose concentration and basal production rate.
- d is a measure of the activated promoter 2 protein production.)

$$x = \frac{\left[P_2^+\right] k_4}{k_4 \left[P_{2,T}\right] + k_{d,A}} \qquad \text{(II-B.12)}$$

$$y = \frac{[A] k_4}{k_4 \left[P_{2,T}\right] + k_{d,A}} \qquad \text{(II-B.13)}$$

$$\tau = t(k_4[P_{2,T}] + k_{d,A}) \qquad \text{(II-B.14)}$$

$$a = \frac{k_4 \left[P_{2,T}\right]}{k_4 \left[P_{2,T}\right] + k_{d,A}} \qquad \text{(II-B.15)}$$

$$b = \frac{k_{-4}}{k_4 \left[P_{2,T}\right] + k_{d,A}} \qquad \text{(II-B.16)}$$

$$c = \frac{k_4 \left(k_3 \left[P_1^+\right] + k_{lk} \left[P_{2,T}\right]\right)}{\left(k_4 \left[P_{2,T}\right] + k_{d,A}\right)^2} \qquad \text{(II-B.17)}$$

$$d = \frac{k_{-4} + k_5 - k_{lk}}{k_4 \left[P_{2,T}\right] + k_{d,A}} \qquad \text{(II-B.18)}$$

If $k_1,k_{-1},k_2,...,k_{d,G},[P_{2,T}] > 0$, ergo, all the constants in the model are positive, the following properties are obtained

$$d > b \qquad \text{(II-B.19)}$$

$$a < 1 \qquad \text{(II-B.20)}$$

$$a, b, c, d > 0 \qquad \text{(II-B.21)}$$

These properties are obtained from $k_5 \gg k_{lk}$ and 2.2.20 from kd,A > 0.

Equilibrium and system stability.

The system is in equilibrium if (In this work x^* and y^* indicate equilibrium values)

$$\frac{bx^*}{a - x^*} = \frac{dx^* + c}{1 - x^*} \qquad \text{(II-C.1)}$$

Reorganizing following solution for x^* is obtained

$$x^*_{1,2} = \frac{ad - b - c}{2(d - b)} \pm \frac{\sqrt{(b + c - ad)^2 + 4ac(d - b)}}{2(d - b)} \qquad \text{(II-C.2)}$$

As $a,b,c,d > 0$ y $d > b$, then

$$(b + c - ad)^2 + 4ac(d - b) > 0 \qquad \text{(II-C.3)}$$

Therefore $x^*_{1,2} \in \Re$ Such that $x^*_1 \neq x^*_2$.

The system will always have 2 real equilibrium states. There is no bifurcation.

As $\frac{\sqrt{(b+c-ad)^2+4ac(d-b)}}{2(d-b)} > 0$, the sign that x∗ takes depends on $\frac{ad-b-c}{2(d-b)}$.

$$\frac{ad-b-c}{2\left(d-b\right)}=\frac{\frac{d}{d}\left(ad-b-c\right)}{2\left(d-b\right)} \qquad \text{(II-C.4)}$$

$$=\frac{d\left(a-\frac{b}{d}-\frac{c}{d}\right)}{2\left(d-b\right)} \qquad \text{(II-C.5)}$$

Being $a<1\wedge\frac{b}{d}<$1 we can approximate

$$a-\frac{b}{d}\approx 0 \qquad \text{(II-C.6)}$$

Thus

$$\frac{d\left(a-\frac{b}{d}-\frac{c}{d}\right)}{2\left(d-b\right)}\approx\frac{-c}{2\left(d-b\right)} \qquad \text{(II-C.7)}$$

Using the same approximation, we get the following result.

$$\sqrt{\left(b+c-ad\right)^2+4ac\left(d-b\right)}\approx\sqrt{c^2+4ac\left(d-b\right)}>c \qquad \text{(II-C.8)}$$

Therefore, if the approximation is valid, it can be assured there is always a positive and a negative state of equilibrium. Only one of these two has a biological interpretation. Based on the next equality

$$\frac{bx^*}{a-x^*}=\frac{dx^*+c}{1-x^*} \qquad \text{(II-C.9)}$$

The positive equilibrium must satisfy $x^* < a < 1$.This will give a maximum value to which the system cannot arrive.

$$x^*<x^*_{max}=a \qquad \text{(II-C.10)}$$

The system equilibrium stability is also studied. And thus, linearization of the differential equation system is necessary. This is done by calculating the differential equation system's Jacobian. The Jacobian of the system *J* is given by

$$J = \begin{bmatrix} \frac{\partial}{\partial x}\left(\frac{dx}{d\tau}\right) & \frac{\partial}{\partial y}\left(\frac{dx}{d\tau}\right) \\ \frac{\partial}{\partial x}\left(\frac{dy}{d\tau}\right) & \frac{\partial}{\partial y}\left(\frac{dy}{d\tau}\right) \end{bmatrix} \quad \text{(II-C.11)}$$

$$= \begin{bmatrix} -y-b & a-x \\ d+y & -1+x \end{bmatrix} \quad \text{(II-C.12)}$$

Be *T* the matrix trace and Δ its determinant

$$T = trace\,(J) = x^* - y^* - (b+1) \quad \text{(II-C.13)}$$

$$\Delta = det\,(J) = x^*\,(d-b) + y^*\,(1-a) + (b-ad) \quad \text{(II-C.14)}$$

Using the same approximation $\frac{b}{d} - a \approx 0$, the following is obtained

$$\Delta \approx x^*\,(d-b) + y^*\,(1-a) > 0 \quad \text{(II-C.15)}$$

$$T = x^* - y^* - (b+1) \quad \text{(II-C.16)}$$

$$T = x - \frac{dx^* + c}{1-x^*} - (b+1) \quad \text{(II-C.17)}$$

$$T = \frac{\left((x^*)^2 + c\right) + x^*\,(d-1)}{x^* - 1} - (b+1) \quad \text{(II-C.18)}$$

$T < 0$ can be proven for all $a,b,c,d,x^* > 0$ and $x^* < 1$.

As long as the approximation used is valid and under the proposed hypothesis, the system will reach a unique stable equilibrium. Figure 4 illustrates a phase plane of the system.

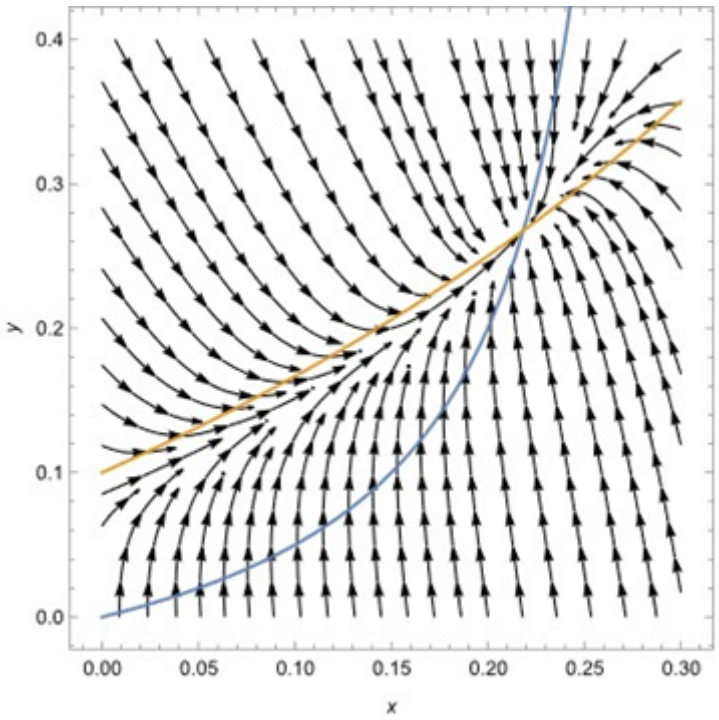


Figure 4. Phase plane of the system with $a = 0.3, b = 0.1, c = 0.1, d = 0.5$.

Cellular automata model

A program based on cellular automata was written using Python which was run on google servers through the Google Colab platform, it does not have a graphical interface and any change in the simulation parameters must be made directly in the code. The program performs a complete update of the cell space applying the rules described below, in all cells of the matrix. Each time step corresponds to a complete update of the matrix and is repeated the number of times that the user determines necessary, remembering that each update cycle corresponds to one minute of time.

The characteristics selected for the model are presented below:

Cell space: It is formed by a two-dimensional square matrix with periodic border, that is, the cells of the opposite border are considered neighbors, of variable size according to the experiment, but which will be as appropriate, with a side equal to 100 or 200 where each matrix cell contains one element, which is identified by a number.

Cells state: The set of states in which the AC cells can be found is given by:

Q = {EV, PrAG, PrA, PAG, PA, PAC, Ar, AC, G, A1}

Table 2. Summary of these that the AC cells can adopt

State	Mean	Numeric identifier in the matrix
EV	Empty space	0
PrAG	Promoter for Activator protein and GFP	1
PrA	Promoter for Activator protein	2
PAG	Activator protein and GFP producer	3
PA	Activator protein producer	4
PAC	AraC producer	5
Ar	Arabinose	6
AC	AraC	7
G	GFP	8
A1	Activator protein	9

Neighborhood: The neighborhood considered in the model is Moore's neighborhood (Figure 5), which, in addition to the orthogonal neighbors, takes into account the neighbors on the diagonals. The use of this type of neighborhood is motivated by the need to provide mobile individuals in the system with the ability to move in any direction.

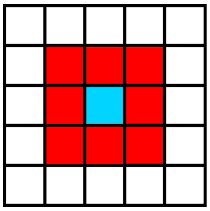


Figure 5. Neighborhood. The blue cell represents the active cell and the red cells their neighbors

Initial configuration: Prior to the execution of the model, the position of the promoters and producers within the simulation area has been pre-established, which will not change with the passage of time.

Time step: Although the execution speed of the program depends on the power of the computer that runs the program, it has been established that the amount of molecules

produced or eliminated in each time step is the same as that corresponding to the step of a minute of real life.

Parameters: The parameters chosen for the execution of the simulation are:

1. Protein synthesis rate
- GFP: 4 molecules per minute
- AraC: 3 molecules per minute
- Activator: 10 molecules per minute

2. Half life
- The half-life changes according to the experiments

Rules of evolution of the model:

1. Cells PrAG, PrA, PAG, PA and PAC will not change their position throughout the simulation
2. EV cell can change position with EV, Ar, AC, G and A1 cells
3. Cells PrAG and PrA check if there are cells A1 and / or AC in your neighborhood
4. Cell PA will randomly transform cells EV to cells A1 at the rate of 10 cells per minute if cell PrA has cells A1 and AC in its neighborhood
5. The PAG cell will randomly transform EV cells to A1 and GFP cells at a rate of 10 and 4 cells per minute respectively if the PrAG cell has A1 cells in its neighborhood
6. The PAC cell will randomly transform EV cells into AC cells at the rate of 3 cells per minute
7. Cells Ar, Ac, G and A1 will degrade according to their half-life

Collision handling: As observed in rule number 2, it is the EV cell that can move through the other cells, that means that it is the empty space that moves between the molecules and not vice versa. This is done to prevent two molecules from trying to occupy the same physical space at the same moment in time.

Parts

Molecular Docking Analysis for Taq / TBD Polymerase.

The amino acid sequence of the modified Taq polymerase underwent modeling on the ROBETTA online server. Which was optimized through the following changes:
A point modification of amino acid 732, which is an aspartic acid in the native protein and changes to asparagine.

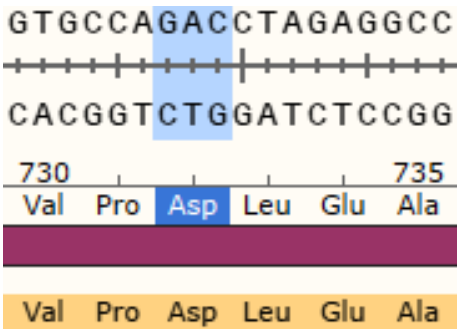


Figure 6. The nucleotide sequence for amino acid 732 (aspartic acid) in the native protein is shown.

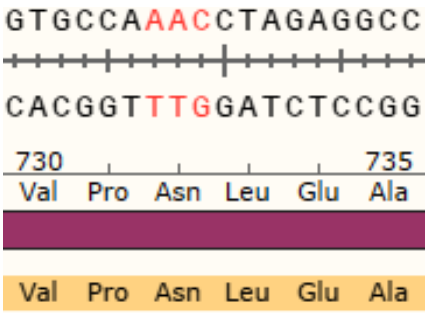


Figure 7. Change in nucleotide sequence for amino acid 732 from aspartic acid to asparagine.

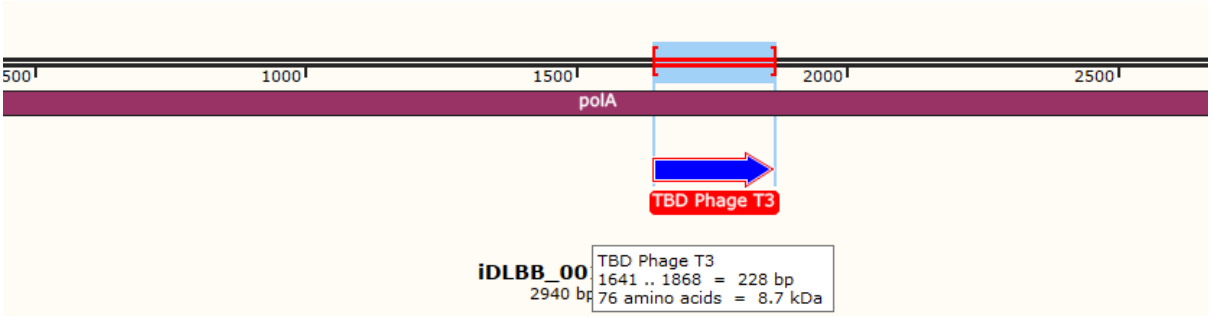


Figure 8. The added sequence encoding the Thioredoxin Binding Domain of phage T3 is shown.

The following figure shows the spatial organization of the sequence after the two modifications have been made in SnapGene ®.

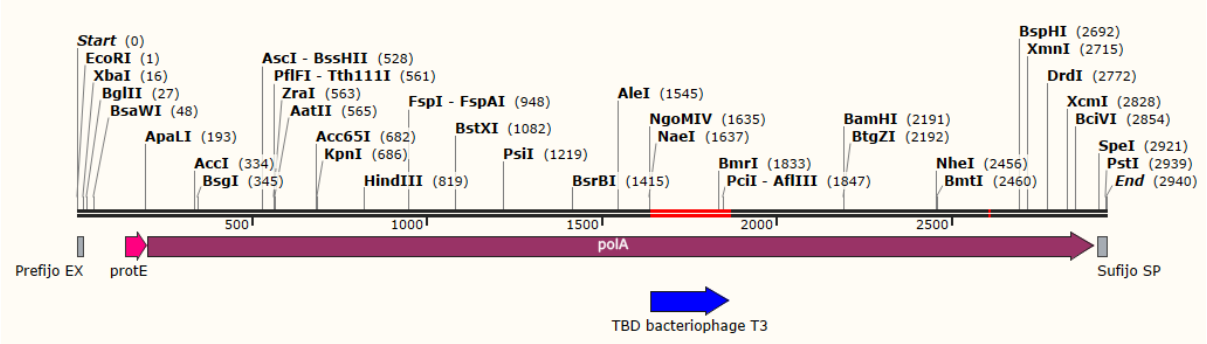


Figure 9. Final construct in SnapGene® for Taq / TBD Polymerase

The protein was modeled by a de novo protein modeling method using the ROBETTA online tool, using the RoseTTAFold method, allowing to know a position of the quaternary structure of the protein.

As a result of the analysis carried out, 5 different predictions were obtained for the folding of the protein, of which the first one was used to carry out the next analyzes, since it is the model that has the least amount of angstroms in estimation according to the same server. ROBETTA. The next step consisted of validating the conservation of some essential structures for the catalytic activity of the protein, for which the UCSF Chimera ® software was used. The following images show a simulation of the modified protein viewed from different angles, highlighting the inserted region.

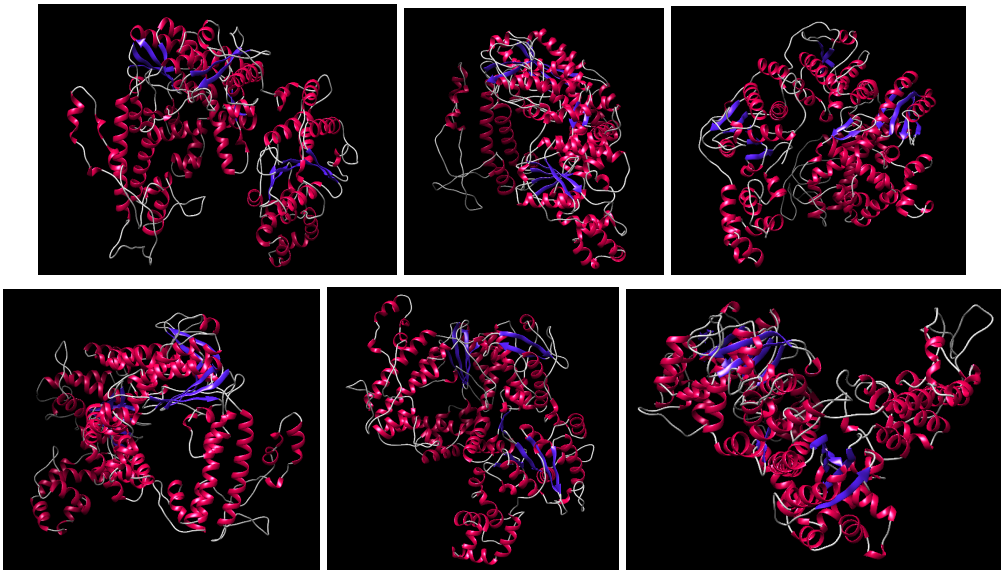


Figure 10. Different perspectives of Taq / TBD Polymerase in UCSF Chimera® software. a) Different perspectives of the protein in the form of secondary structures helices and sheets

After checking in the software from different angles, the Taq / TBD polymerase and the Taq Polymerase Wild Type (1TAQ) were superimposed, which was obtained from the Protein Data Bank database, as shown below. This superposition was made to be able to visualize

the level of structural modification suffered by the protein and for a later analysis and evaluation of the effects of the modification on the catalytic regions.

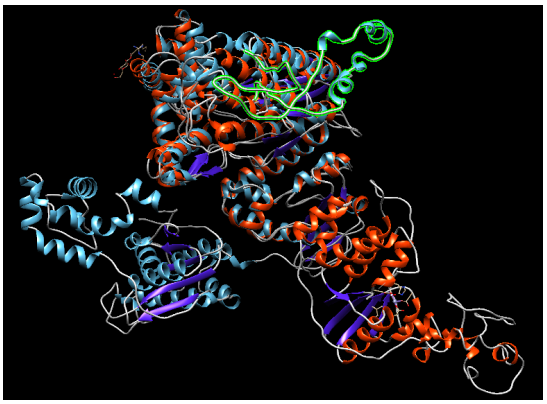


Figure 11. Superposition and alignment of Taq / TBD Pol structures (cyan) and Taq Pol wild type (orange) by the Clustal Omega Alignment method, thioredoxin binding domain, (cyan and green outline).

When visualizing the splicing of the proteins, it is appreciated that the original structure of the protein is conserved in a considerable way, including the catalytic region, it is possible to find a correspondence in the upper part where the catalytic domains are found, however, it is possible to observe a shift in them for 5 'nuclease activity for both proteins, which are the lower part, where the first 300 amino acid residues can be found.

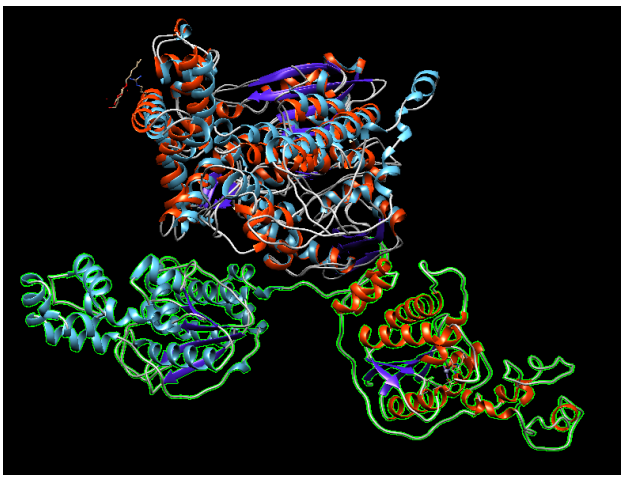


Figure 12. 5 'nuclease activity domains for Taq / TBD (cyan) and Taq Pol WT (Orange) This displacement is considered to be due to the addition of the sequence for TBD that causes the displacement of the domain for nuclease activity, TBD displaces the other domain by some type of interaction. However, it can be seen that the structures are preserved.

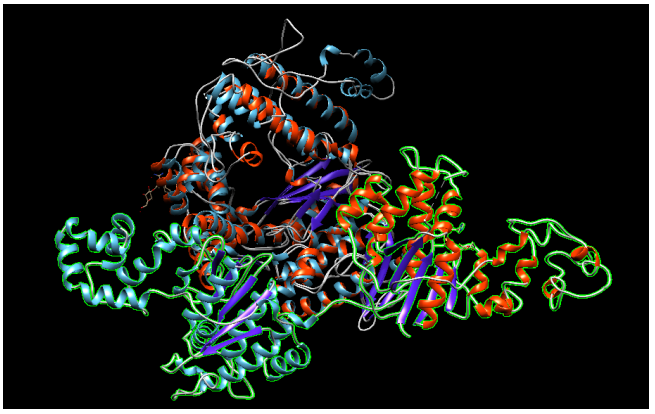


Figure 13. Perspective view of Taq / TBD Pol and Taq Pol WT

Although these domains are not aligned, the previous explanation for this was determined, finding a correspondence for both domains, under this statement, that the structure is correlated to function, it can be asserted that the 5 'nuclease activity will be present in the protein modified.
Likewise, this assertion is considered for the other domains of the protein, as shown in the following figures:

Figure 14. Domains for 3-5 'exonuclease activity at residues 294-422.

Figure 15. Domain for polymerase activity, residues 424-831 (Kim, Y., 1995), in this range the thioredoxin binding domain of residues 480-555 is included.

In the two previous images, clear correspondences of the domains between both proteins are observed, again we can deduce that the corresponding functionalities will be conserved in the Taq / TBD Polymerase. It is interesting to note that the thioredoxin-binding domain is the only exception to this alignment, and remembering what was mentioned for this domain,

it is expected that it is within the domain that refers to polymerase activity, since its function is to confer an improvement in processivity and fidelity in DNA synthesis.

Given the above characteristics, we could assert that DNA synthesis would not be affected since the domain is observed intact in the splicing regions, except for TBD, we find a clear correspondence in the anchoring site of the ligand in the protein, which in this case is the DNA strands.

After obtaining, modeling and comparison of structures in UCSF Chimera ®; Docking or molecular coupling analyzes of the protein-nucleic acid type were carried out, in this case being Taq / TBD Polymerase with DNA / RNA in the HDOCK molecular coupling and modeling online server.

HDOCK is an integrated multi-component package that includes various third-party programs, this coupling algorithm and scoring functions, and a host of tools developed by those who have implemented the server.

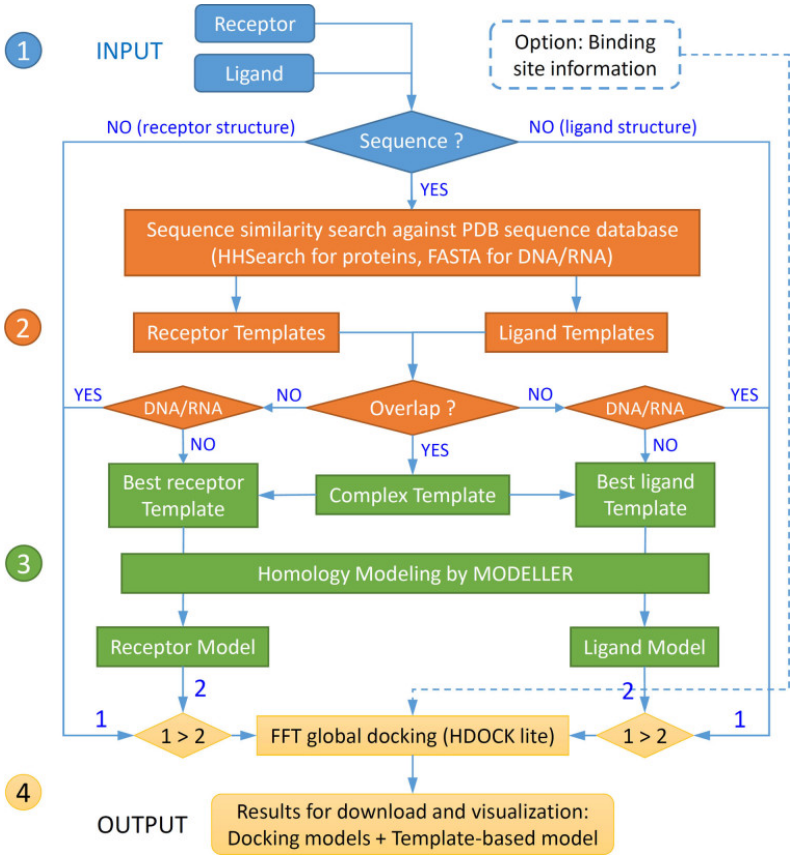


Figure 16. The workflow of the HDOCK web server is divided into four stages: (1) data input, (2) sequence similarity search, (3) structure modeling, and (4) FFT-based global docking in which priority is given to user-input structures.

HDOCK web server workflow is divided into 4 stages

Data entry: is data entry that accepts both sequences and structures for proteins.

Sequence Similarity Search: Given the input sequences or converted from structures, a sequence similarity search is performed against the PDB sequence database to find the

homologous sequences for receptor and ligand molecules. For proteins, the HHSuite package is used for sequence searching. For DNA / RNA, the FASTA program is used, as it is robust and easy to use for searching protein and DNA / RNA sequences. Generally, this apso will produce two sets of homologous templates (one for receptors and one for the ligand).

Structure modeling: In this step we will be comparing two sets of templates to see if they have common records with the same codes in PDB. If such PDB codes exist, a common template will be selected for both the receptors and the ligand. However, in this case there is no overlap between two sets of homologous templates, the best templates for the receptor protein and ligand will be selected from two sets of templates. If multiple templates are available, the one with the highest sequence coverage, the highest sequence similarity, and the highest resolution will be selected. In addition; a complex template is given priority over an apo structure, during template selection, if the differences between each template are within 10% of sequence coverage, similarity, and resolution. With the selected templates, the models are built using MODELLER, in which the alignment sequence is performed using ClustalW.

Molecular docking: With the structures modeled by the server or uploaded by the users, the workflow enters the last step, the molecular docking. At this point, the server uses HDOCKlite, a hierarchical FFT-based coupling program, which is used to globally sample putative link orientations. The linking process will also incorporate the information from the binding site if users have provided such information at the time of submission.

The HDOCKlite docking models and the MODELLER template-based model are provided interactively for users to download via a web page and email notification. On the results page, users can also view the top 10 docking models through a Jmol web interface.

The molecular docking analyzes were carried out on the previous server sending both the Taq TBD polymerase and the Wild Type Taq polymerase sequence in order to compare their interactions with DNA.

Results

Taq polymerase molecular docking.

Below are some images that will serve to visualize the results obtained for Taq / TBD polymerase 3D-modelling and molecular docking, as well as docking scores and ligand interactions.

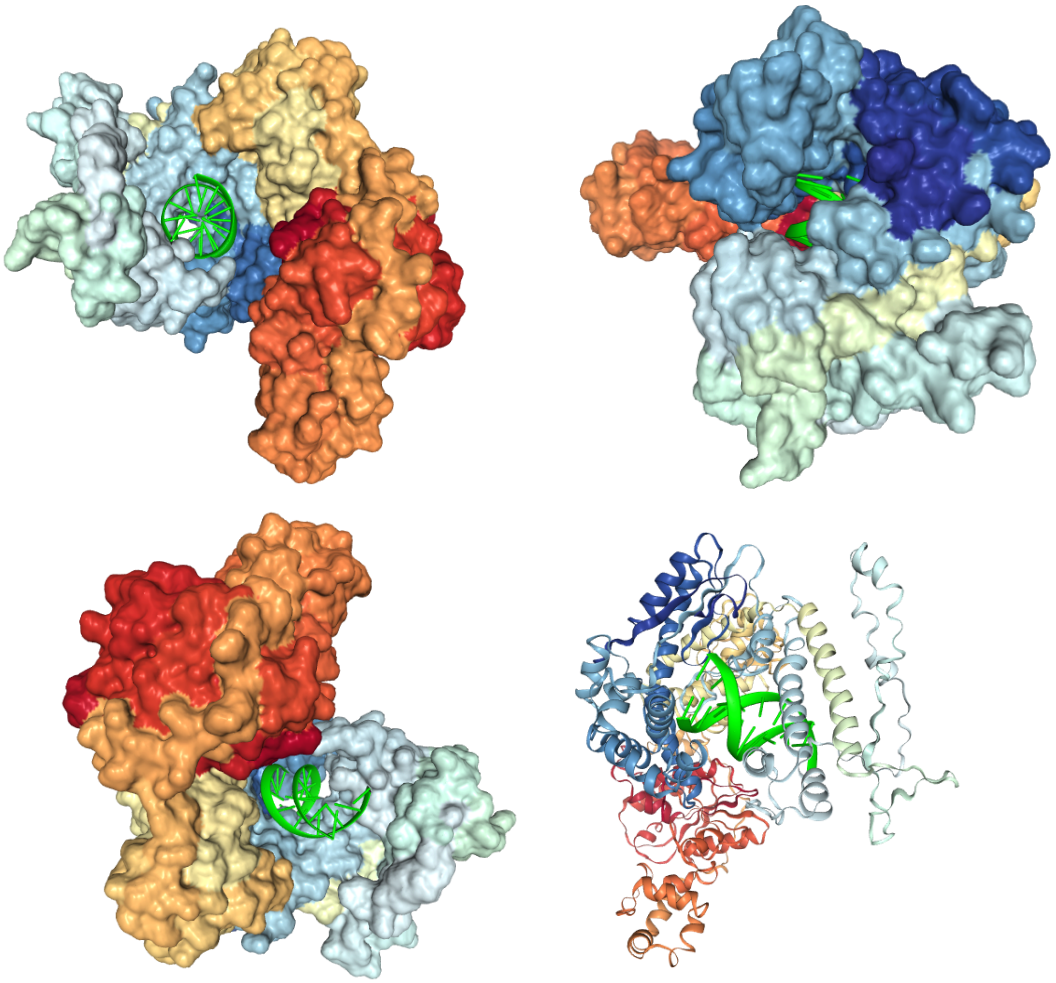


Figure 17. Molecular docking results for Taq / DNA polymerase receptor and DNA ligand.

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-289.99	-270.52	-270.10	-263.33	-260.02	-251.22	-250.50	-250.09	-249.05	-246.35
Ligand rmsd (Å)	53.69	50.35	8.59	57.50	50.35	52.67	55.35	19.81	51.74	52.57

The previous images show the results of the protein-ligand molecular coupling, in which an interaction between both molecules can be observed, in this case model number 2 is shown, since it is the one found in the catalytic domain identified as the one that carries out polymerase activity. However, model number 3 also shows an association, specifically in the domain identified with 5 'nuclease activity, as can be seen in the following figures.

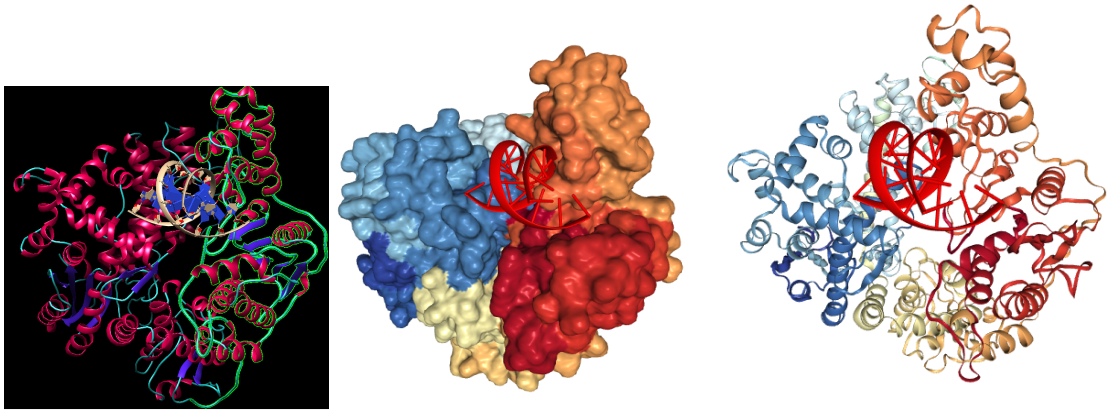


Figure 18. Comparison of model number 3 in UCSF Chimera ® (left) where the domain involved in the 5 'nuclease activity is indicated and in HDOCK in surface format (center) and in the form of secondary structures (right).

In the previous illustration, the association that results in the domain for 5 'nuclease activity is observed, a correspondence with the domain found in the literature with the one that results in the coupling in HDOCK is observed, as shown in the models that show the secondary structures , corresponds to the coupling of the DNA with the designated domain.

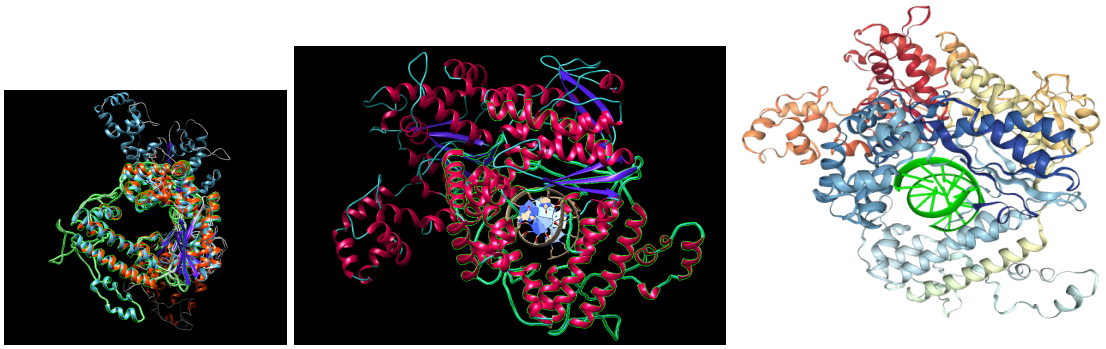


Figure 19. Comparison between structures in UCSF Chimera ® without ligand (top left) and with ligand (top right) in the domain involved in polymerase activity with the coupling obtained in HDOCK.

The previous images show the comparison between the models for Taq / TBD Polymerase in the domain related to DNA polymerization activity, a conservation of this domain is observed if it is compared with the image where the superposition of the enzymes is visualized.

Regarding the analysis of the domains related to the three activities mentioned for the modified enzyme, the results obtained for the exonuclease 3'-5 'activity are found, for which the following illustrations are shown.

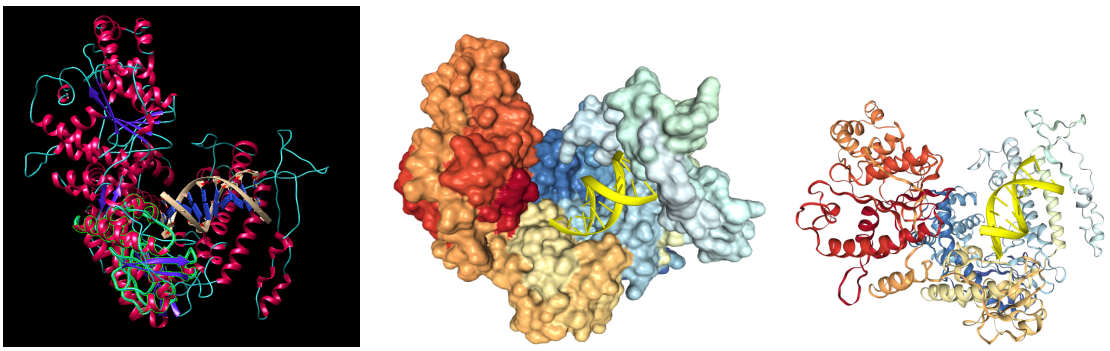


Figure 20. Comparison of structures in UCSF Chimera ® and HDOCK in perspective of amino acids 294 to 422 involved in exonuclease activity 3'-5 '.

The illustrations above seek to show the correspondence that exists in the 3-5 'exonuclease domain of activity. In the UCSF Chimera ® image, the domain reported with this exonuclease activity is indicated with a green outline. you can verify correspondences in the models and the designated area.

To finish with the activities related to DNA, several figures are included below with the intention of visualizing some similarities and correspondences between the binding sites obtained in HDOCK for Taq / TBD polymerase and comparing them with the Wild Type Taq polymerase.

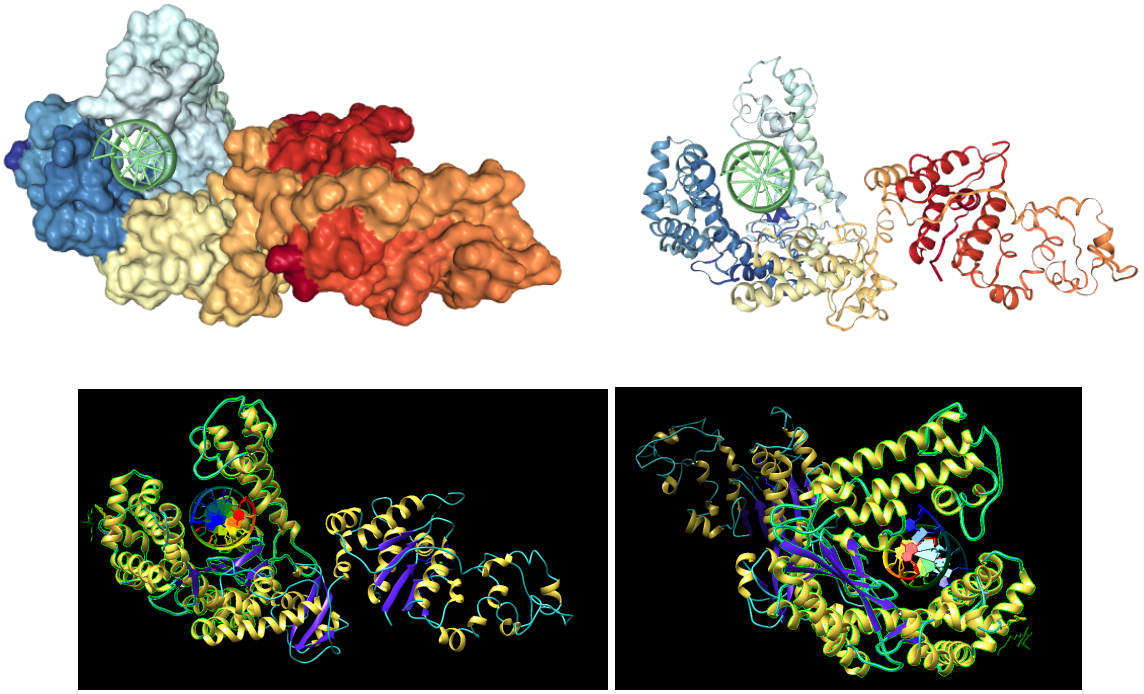


Figure 21. Structures in HDOCK (top) and in UCSF Chimera ® (bottom) for Wild Type Taq Polymerase indicating the domain related to polymerase activity.

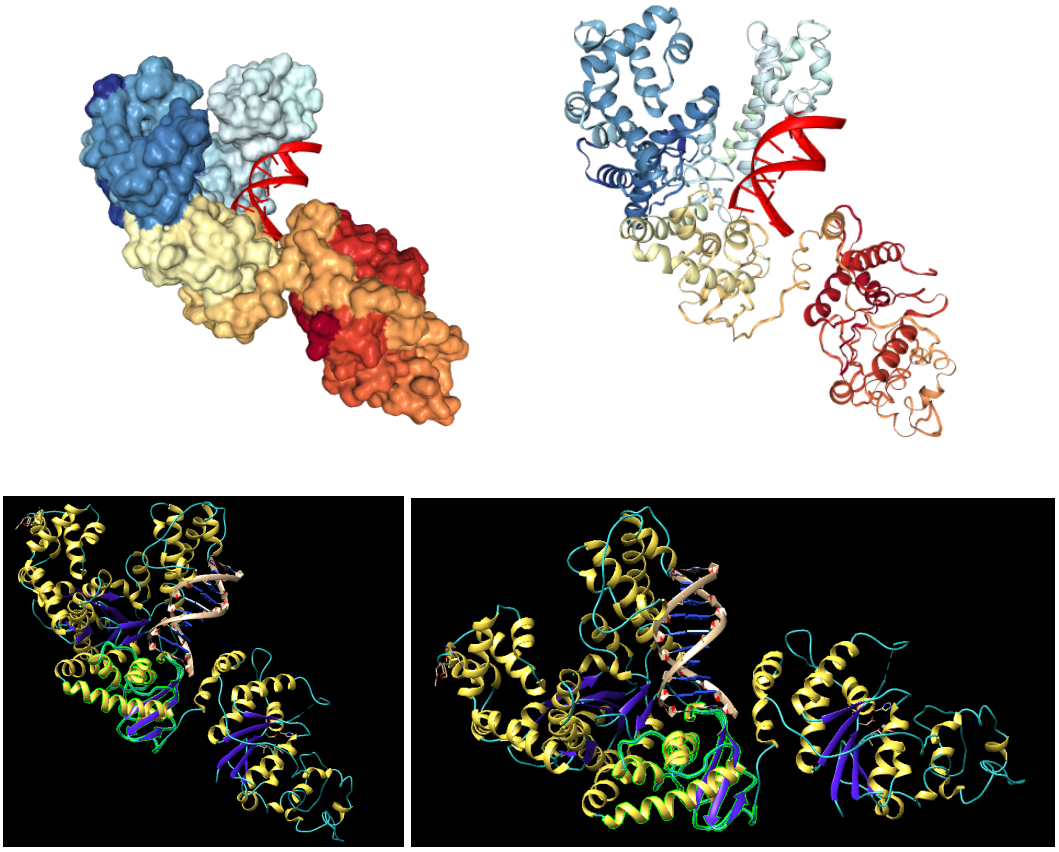
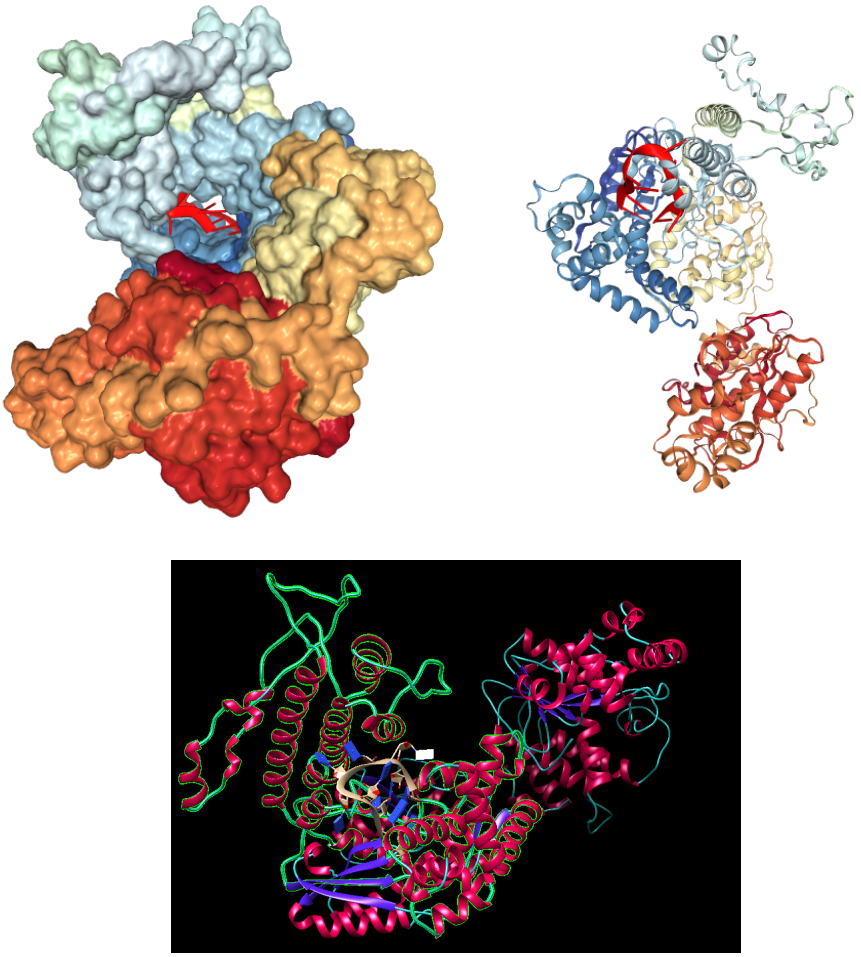


Figure 22. Structures in HDOCK (top) and in UCSF Chimera ® (bottom) for Wild Type Taq Polymerase indicating the domain related to 3'-5 'exonuclease activity.

In the previous images, the domain involved in the polymerase activity for the Wild Type Taq Polymerase (amino acids 424-831) and for 3'-5 'exonuclease activity (amino acid 294-422) is indicated where, as for Taq / TBD pol, DNA is located in the designated domain with the same amino acids, which are consistent with the overlaps made earlier.

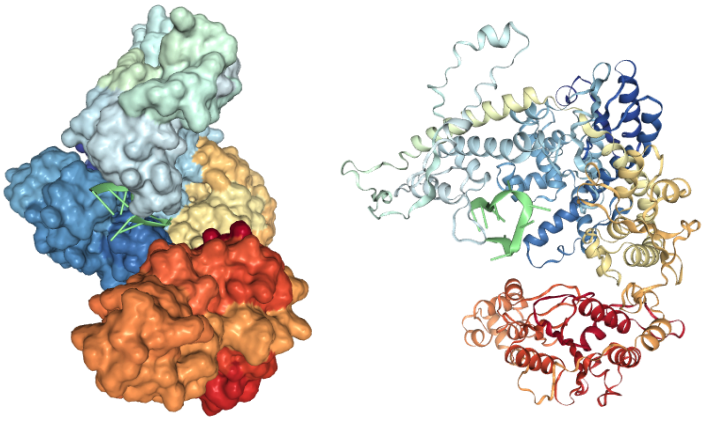
This indicates that there is a correspondence between the structures and their proposed functions, which is a good indication for the purposes for which the chimeric protein Taq / TBD polymerase was designed. However, in the coupling results obtained for the wild type enzyme, it was not possible to find any model that predicted the interaction between DNA and the first 300 amino acids, involved in the 5 'nuclease activity, therefore, a group of images in this last comparison. Even so, with the interactions shown in Taq / TBD polymerase and DNA in that domain, they agree with that reported in the literature, together with the rmsd values for ligand and docking score, a fairly favorable interaction is expected in this domain and the DNA for the enzyme Taq / TBD polymerase

To conclude with the molecular coupling analyzes, the Docking analysis for Taq / TBD Polymerase and RNA was carried out, in which the following results were obtained.



Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-319.61	-302.84	-272.26	-269.95	-266.20	-262.64	-257.57	-256.67	-254.75	-252.68
Ligand rmsd (Å)	31.49	33.34	36.69	32.18	30.63	33.58	34.28	38.19	25.58	30.61

Figure 23. Results for model number 3 of the coupling between Taq / TBD Polymerase and RNA.



Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-319.61	-302.84	-272.26	-269.95	-266.20	-262.64	-257.57	-256.67	-254.75	-252.68
Ligand rmsd (Å)	31.49	33.34	36.69	32.18	30.63	33.58	34.28	38.19	25.58	30.61

Figure 24. Results for model number 9 of the coupling between Taq / TBD Polymerase and RNA.

As can be seen, there is an interaction in the same area where the polymerization reaction occurs for both models of coupling between the enzyme and the DNA; and as in these results, the others are located in the same neighborhood. With the above it is possible to have an approach and also to expect a reverse transcriptase activity.

In many of the results, it is possible to notice the presence of the parameter called Ligand rmsd (Å), which is called the deviation of the root mean square of the atomic positions, or simply the mean square deviation (RMSD), which is the average measure between the atoms of the molecules evaluated, in this case receptor and ligand; Taq / TBD polymerase and DNA or RNA respectively. It is recommended to use models that have a value less than 5; if they are between 1 and 3, it is better. Although relatively high values are found compared to those proposed.

Differential equation numerical solution.

Next, numerical solutions for the differential equations system in various initial conditions are graphed(see fig. 25, 26 y 27). Ode45 solver from MATLAB®was used to solve them. Parameters and units were chosen arbitrarily:k_4 = 0.8;k_{-4} =0.4;k_3 = 8;$[P_{1,T}]$ = 8;$[Ara]$ = 1000;H = 0.2;n = 2;k_{lk} = 0;$[P_{2,T}]$ = 8;k_5 = 3;$k_{d,A}$ = 8;$k_{d,G}$ = 0.4.

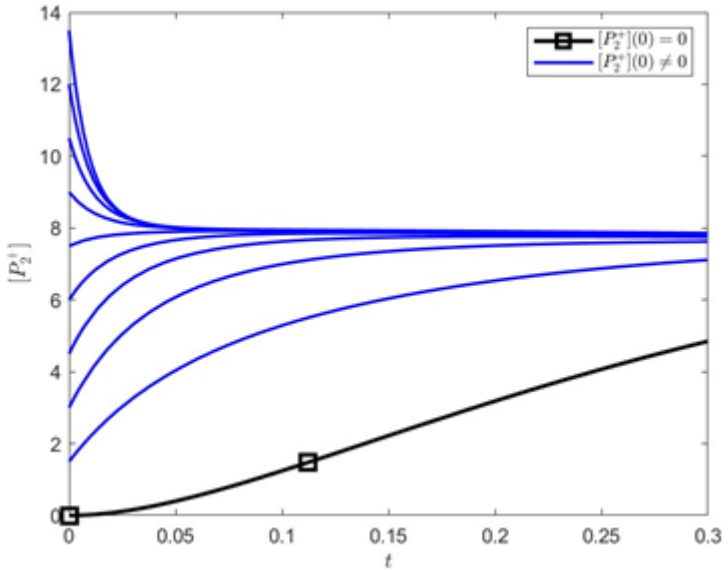


Figure 25. Activated promoter 2 concentration dependent of time with various initial conditions.

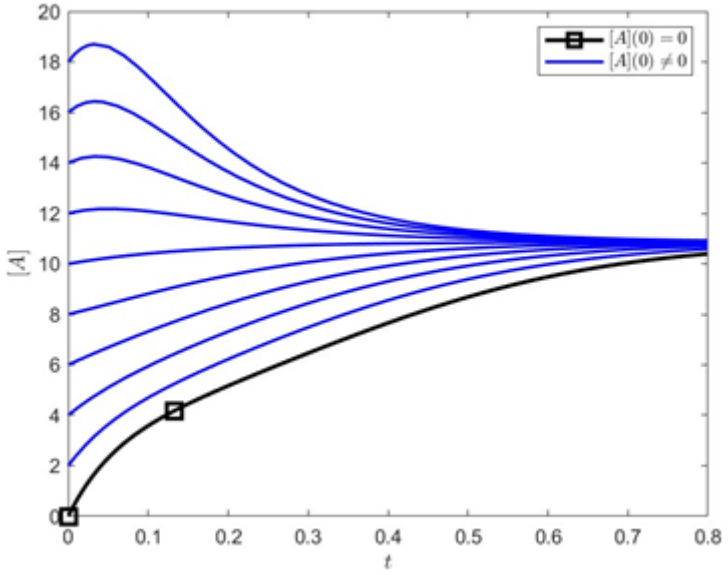


Figure 26. Activator protein concentration dependent of time with various initial conditions.

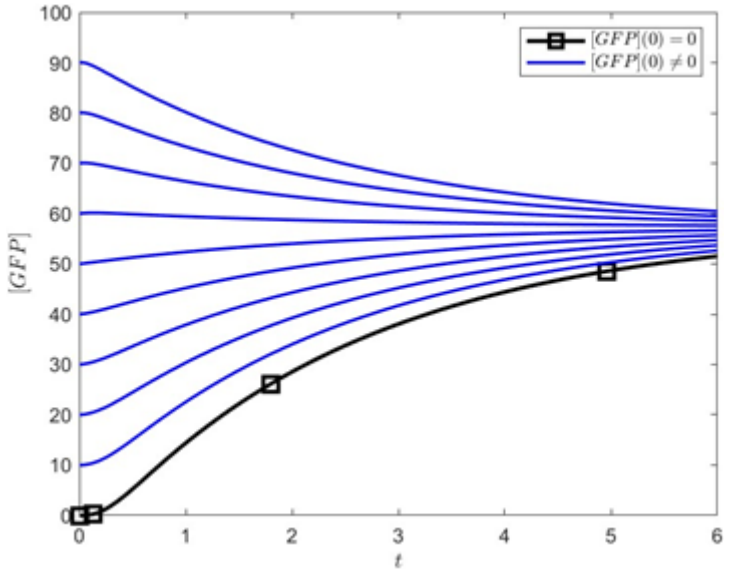


Figure 27. GFP protein concentration dependent on time with various initial conditions.

Hysteresis.

Memory or hysteresis is one of the main characteristics of this genetic circuit. Once the inductor is added, the circuit remembers its active state. The target protein will be produced even after degradation of the inductor sugar, keeping the circuit in active state. The differential equations system can model this behavior: As $[P_1^+]$ and klk equal to zero then c = 0. All the previous properties are still true: a,b,d > 0,d > b,a < 1. Substituting c = 0 on the x∗ equilibrium solutions

$$x_{1,2}^{*}=\frac{ad-b-c}{2\left(d-b\right)}\pm\frac{\sqrt{\left(b+c-ad\right)^{2}+4ac\left(d-b\right)}}{2\left(d-b\right)} \quad \text{(II-E.1)}$$

$$x_{1,2}^{*}=\frac{ad-b}{2\left(d-b\right)}\pm\frac{b-ad}{2\left(d-b\right)} \quad \text{(II-E.2)}$$

$$x_{1}^{*}=0 \quad \text{(II-E.3)}$$

$$x_{2}^{*}=\frac{ad-b}{d-b} \quad \text{(II-E.4)}$$

It is known that *d* > *b*, so for a big enough *a* we can assume *ad* > *b*. Then 0. Two equilibriums with biological significance exist, a positive one and one equal to 0. Now the stability of these two equilibria is studied. When x^{*} = 0 and y^{*} = 0.

$$T=-\left(b+1\right)<0 \quad \text{(II-E.5)}$$

$$\Delta=b-ad<0 \quad \text{(II-E.6)}$$

This means this equilibrium is a saddle point and thus unstable. When x^{*},y^{*} > 0

$$T<0 \quad \text{(II-E.7)}$$

$$\Delta=y^{*}\left(1-a\right)>0 \quad \text{(II-E.8)}$$

This implies equilibrium here is stable. The phase plane in 7 is an example. Two equilibriums can be observed (stable x^{*},y^{*} > 0 and unstable x^{*},y^{*} = 0).

While the basal production is negligible, and arabinose has no been added the system is in unstable equilibrium where the concentrations of [P_2^+], arabinose and GFP are 0. Nevertheless, a fluctuation in the system can take it to its stable equilibrium, where the concentrations are no longer zero. When the system is on a stable equilibrium state where the concentrations are greater than zero, if the arabinose degrades in its entirety the system will reach a new equilibrium state such that $x*,y* > 0$. The system remembers its state, this phenomenon it's called hysteresis. The hysteresis can be visualized with a (see fig. 29) obtained via numerical solutions and variation in the arabinose concentration.

The parameter [Ara] > 0 is changed to [Ara] = 0 around the time unit ≈ 19, to mimic arabinose depletion. The system then seeks a new stable state where the GFP concentration diminishes but never becomes 0.

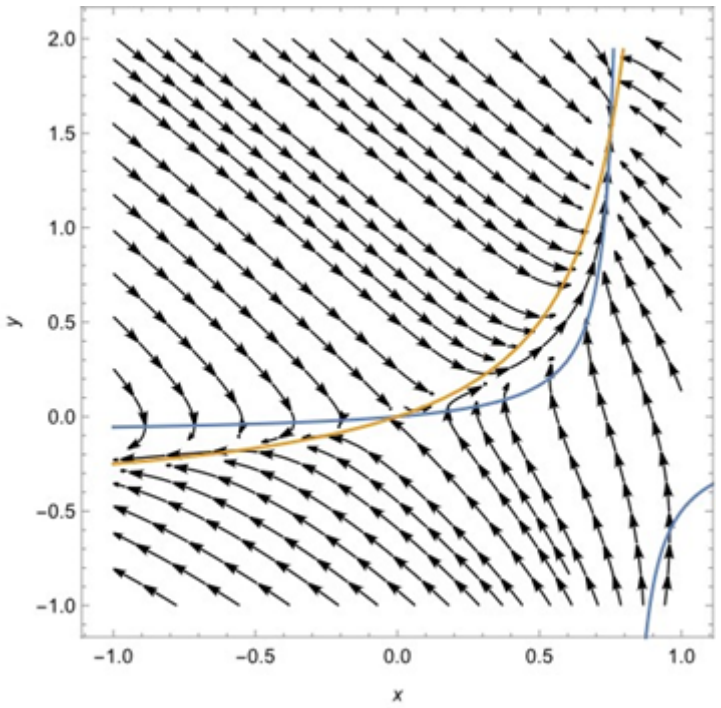


Figure 28. Plane with 2 equilibria. The values are $a = 0.8, b = 0.1, c = 0, d = 0.5$.

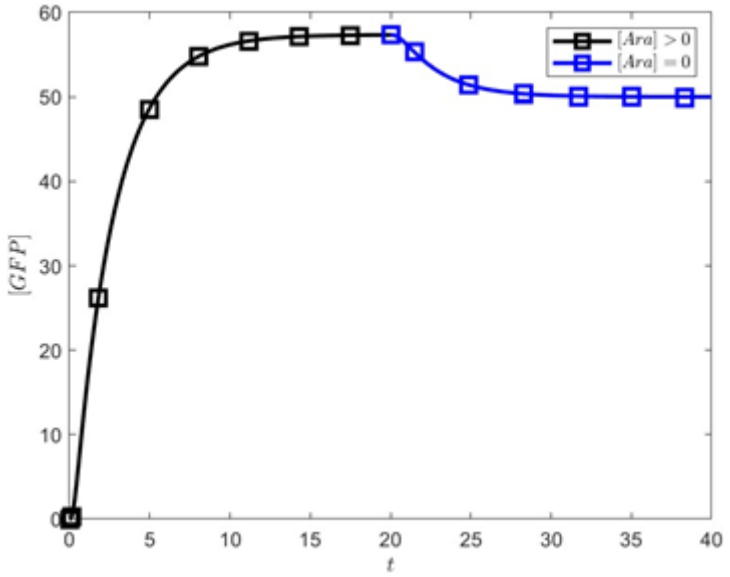


Figure 29: GFP protein concentration dependent of time varying arabinose concentration. This graph shows the circuit's memory (hysteresis).

Prediction of target protein concentration.

It is necessary to be able to make predictions of the target protein (GFP) concentration for this circuit. Also knowing if this concentration is dependent with the initial concentration of the inductor (arabinose). Since the equilibrium GFP concentration is the maximum we can achieve, the following case becomes of interest:

$$\frac{d[GFP]}{dt} = 0 \qquad \text{(II-F.1)}$$

Substituting on 2.2.6 and reorganizing

$$[GFP]_{ss} = \frac{k_5}{k_{d,G}}[P_2^+] + \frac{k_{lk}}{k_{d,G}}[P_2] \qquad \text{(II-F.2)}$$

Where $[GFP]_{ss}$is the equilibrium concentration of GFP. It is also known that $[P_{2,T}] = [P_2^+] + [P_2]$, substituting and reorganizing.

$$[GFP]_{ss} = [P_2^+](\frac{k_5 - k_{lk}}{k_{d,G}}) + \frac{k_{lk}}{k_{d,G}}[P_{2,T}] \qquad \text{(II-F.3)}$$

It becomes known here that the equilibrium concentration of GFP is a linear function of the activated promoter 2 concentration [P_2^+]. It is sufficient to establish which variables rule the equilibrium concentration. This comes from:

$$x_{1,2}^* = \frac{ad - b - c}{2(d-b)} \pm \frac{\sqrt{(b+c-ad)^2 + 4ac(d-b)}}{2(d-b)} \qquad \text{(II-F.4)}$$

As studied, the 0 approximation will be used to prove the equilibrium's stability. This approximation allows a simpler expression for x^*.

It is clear that *c* is an arabinose concentration related constant. Thus, both expressions can be graphed to get a notion of the relationship between x^* and *c* and hence, between $[GFP]_{ss}$ and [*Ara*].(see fig. 30)

Regardless of the chosen values, the behavior of x^* becomes rapidly independent of *c* while this value increases. This reinforces the hypothesis that the circuit's behavior is nonlinear for arabinose, meaning, low arabinose levels can trigger high concentrations of interest protein. The circuit may behave in a “All or nothing” type of way regarding arabinose. Carrying out experiments becomes necessary to determine the minimum concentration of arabinose needed.

This allows the model to move away from the dependency that [*GFP*] and [*Ara*] may have, thus developing a more useful expression. Back to equation 2.6.3,it is valid to assume that in equilibrium [$[P_{2,T}] \approx [P_2^+]_{ss}$], the equation is reduced to a (Assuming klk ≈ 0 leads to the same result.)

$$x^* = \frac{1}{2(d-b)}(\sqrt{c^2 + ac(d-b)} - c) \qquad \text{(II-F.5)}$$

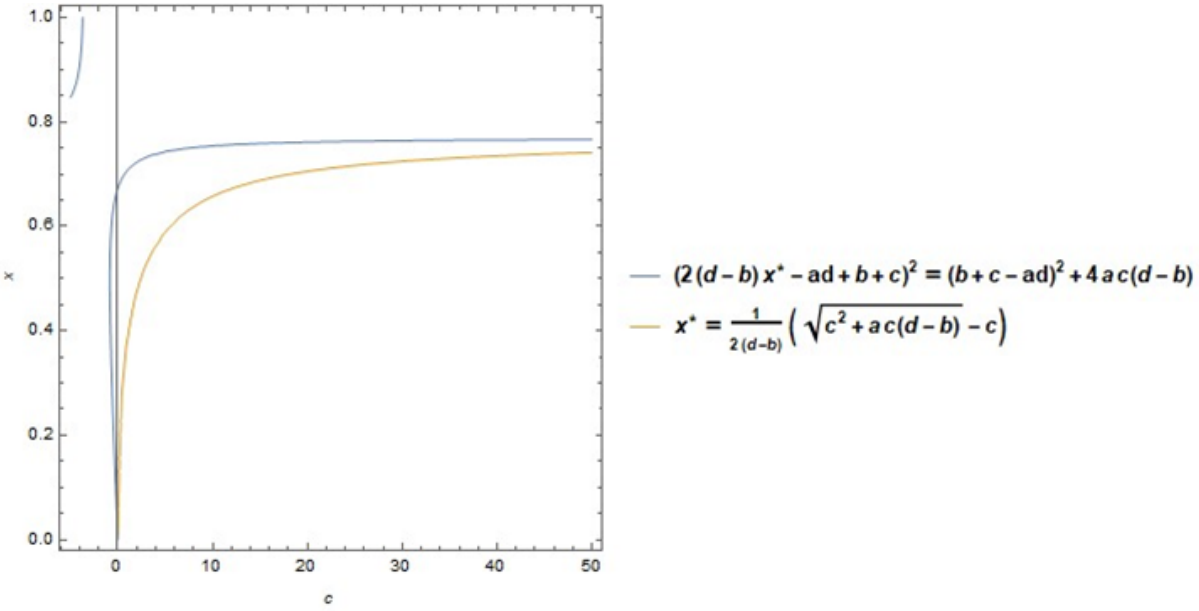


Figure 30: x^* dependency with *c*, reflecting $[GFP]_{ss}$ dependency with [*Ara*].(*a* = 0.77,*b* = 1.1,*d* = 3.7)

$$[GFP]_{ss} = [P_{2,T}](\frac{k_5}{k_{d,G}}) \qquad \text{(II-F.6)}$$

The plasmid concentration containing promoter 2 [$P_{2,T}$] has been considered constant in this model. It can be assumed, in a different time scale, that it follows a Luedeking-Piret type kinetic.

$$\frac{d[P_{2,T}]}{dt} = \alpha\frac{d\chi}{dt} + \beta\chi \qquad \text{(II-F.7)}$$

Where χ is cell concentration, α and β are constants of the model related with the synthesis of the metabolite. Plasmid production is solely associated with cellular growth, meaning $\beta = 0$, and consequently

$$\frac{d[P_{2,T}]}{dt} = \alpha \frac{d\chi}{dt} \qquad \text{(II-F.8)}$$

$$[GFP]_{ss} = \frac{k_5 \alpha}{k_{d,G}} \chi(t) \qquad \text{(II-F.9)}$$

Cellular automata

General behavior of the system

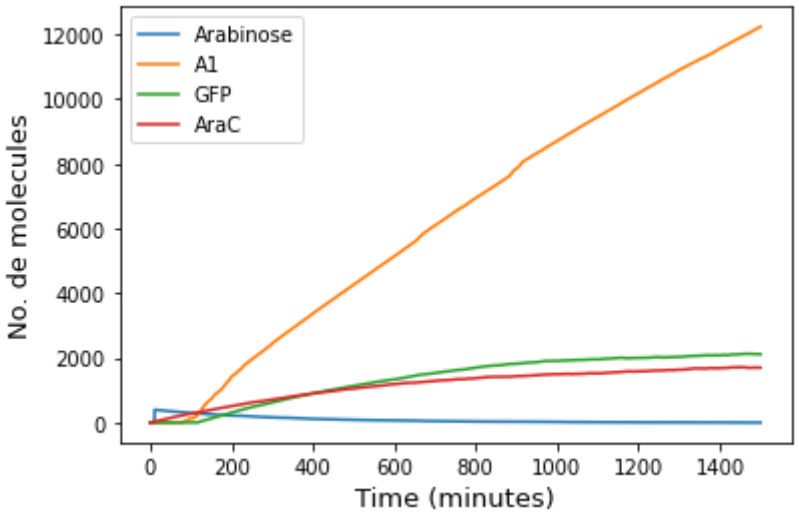


Figure 31. Simulation of the general behavior of the system, a 200-side matrix was used, half-life of the activator protein of 1200 minutes and half-life of arabinose in 200 minutes.

In the general behavior of the system we can observe expected results, AraC is produced constitutively and does not accumulate, contrary to the activating protein, which, when produced by two promoters, reaches large amounts within the cell.

Activator protein accumulation

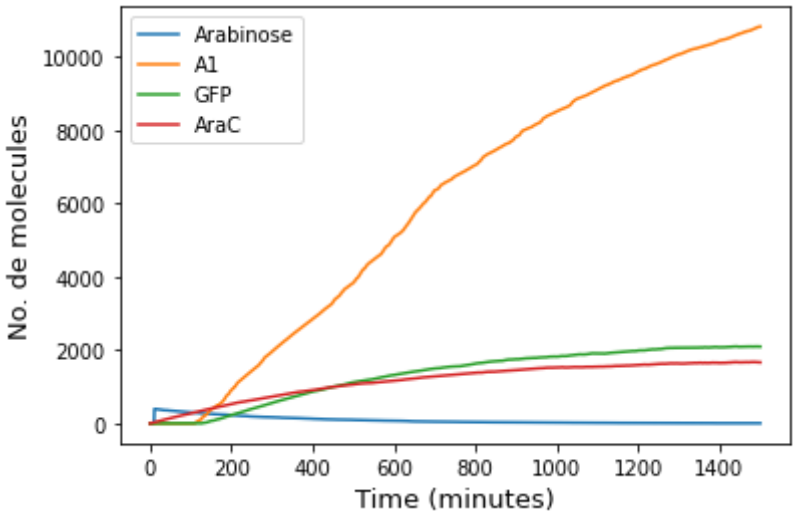


Figure 32. The half-life of the activating protein has been reduced to half (600 minutes), this can be done by adding a degradation tail to the protein, however, its accumulation is still considerably high

In the general behavior of the system (Figure 31), a large accumulation of activator protein is observed even when arabinose is degraded. One of the possible solutions is to add an amino acid sequence that promotes the degradation of the protein, which is known as the degradation tail. Figure 32 shows the development of the simulation with the degradation tail, which decreases, on average, the half-life of arabinose by half. The result is unfavorable, the amount of activating protein is still excessive, which could be a problem for the cell, both due to energy consumption and the stress that the accumulation of this protein could cause in the cell.

There are other control methods by which the amount of activator protein produced by the cell could be reduced, for example, changing the consensus sequences of the promoters or the ribosome binding site, which could considerably reduce the use of cellular resources since reducing the half-life of the protein only makes it degrade more quickly, without causing changes in its production rate, so they use the same resources for its synthesis. By making the changes in the aforementioned sequences, the speed of production of the activating protein is regulated, thus reducing its amount and therefore the amount of resources that the cell spends in its production.

Self-activation capability

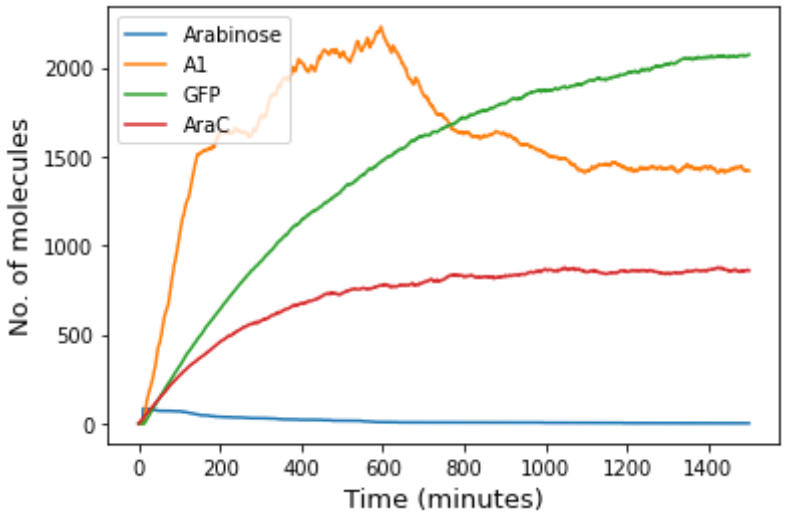


Figure 33. By reducing the half-life of arabinose to 100 minutes and maintaining that of the activating protein at 50 minutes, it is more evident that the memory mechanism is effective

To show that the self-activation mechanism is effective in the event that the half-life of the activating protein was much shorter than expected, a simulation was run in which the half-life of the activating protein was small together with rapid degradation. of arabinose, as observed in figure 33. At the beginning of the simulation, a rapid rise is observed in the amount of activating protein given by the induction of arabinose, as well as auto-induction, as the amount of arabinose is consumed. Activator protein decreases until it appears to reach a stable value, even with arabinose already absent.

Visit https://github.com/OllinSynBioIPN/TETL-BOX to see all the code used in this work.

Conclusions

Taq-TBD molecular docking.

Based on the predictions shown by the previously described models for the Taq polymerase, we expect that in the near future; When it is possible to carry out the evaluation of the protein with laboratory tests, favorable results will be obtained that validate the modifications implemented.

Likewise, in all the results made and shown previously; It is possible to visualize values less than 0 in the coupling scores, this is an indication that; at least in silica, there is an association between Taq / TBD, DNA and even RNA; Since the row corresponding to the Docking Score indicates how spontaneous the union between the two molecules is, it can be confirmed by means of this coupling analysis, based on the change in Gibbs free energy, which is a thermodynamically favorable process, since it is spontaneous by having a docking score less than zero.

The analysis carried out previously and the results obtained; confirm, at least in silico a great possibility that the reactions are carried out, the confirmatory tests regarding the improvements in processivity, fidelity, efficiency and even the reverse transcriptase activity, must be carried out in a laboratory, however, these analyzes They serve as a preamble as to what to expect then.

Cellular automata

Despite the drawbacks produced by the uncertainty in the value of the half-life of the activator protein, the production of GFP is satisfactory in all cases, it is even interesting to see how it seems to reach the same value in all three situations, which indicates that self-activation is possible even at low concentrations of activator protein. It is imperative, then, to know experimentally the rate of degradation of such a protein in order to choose whether or not to apply some method to reduce its concentration and metabolic stress for the cell, especially considering that the assigned half-life corresponds to the average of all the proteins and that could not be realistic due to factors not considered in the simulation of the degradation rate, such as the size of the protein, not having other functions in the cell, presence of sequences that could affect its degradation rate, etc. Still, the simulations make it clear that self-activation with small amounts of arabinose is possible and eliminates the need for other, more expensive compounds, such as IPTG.

Mathematical model

Thanks to the proposed hypothesis (AraC and Arabinose in equilibrium), the differential equations model yields many interesting results. These will help predict the circuit's real behavior. It is known now that the whole system dynamics can be understood by solely analyzing the dynamic between activator protein and activated promoter 2. This is due to the target protein (GFP) equilibrium concentration being a lineal function of the activated promoter 2 concentration P_2^+]). Therefore, the [P_2^+] concentration is a reflex of the GFP concentration. Thanks to this a 2 equation non-dimensional system was obtained. Analyzing equilibrium states and via a justified approximation, it was proven that activator protein, [P_2^+] and GFP concentrations will arrive at a stable value in time while arabinose is present. This value will depend on system parameters (transcription factors bonding and dissociation force, protein degradation and production rate constants, plasmid concentration, arabinose concentration). At the same time this value will not be a function of its initial concentration presence of GFP, activator protein or [P_2^+] does not matter and the equilibrium concentration will always be reached. To exemplify this proven results, numeric solutions of activator protein, GFP and [P_2^+] concentrations were run. It was observed that initial conditions do not matter, and an equilibrium state will be reached. All the curves converge in a single value (see fig. 25, 26 y 27) Equilibrium and system stability changes if there is no inductor presence and basal production is negligible. In this case, 2 equilibrium states were proven: First, when there is no GFP, activator and [P_2^+] production. The system is in OFF state and the equilibrium is unstable. In the second

equilibrium GFP, activator protein and [P_2^+] concentrations are positive and different from 0. This is the system's ON state, and it is stable. This can be summarized as: • The system will be OFF exclusively if there is no arabinose, activator protein and [P_2^+] presence and basal production is negligible. Any fluctuation on the system can take it to its ON state.

- If the system is ON, it can not go back to an OFF state. Even after arabinose is completely exhausted, the system will maintain its ON state (hysteresis). This does not mean the ON states with and without arabinose have the same equilibrium values. The ON state without arabinose may produce less target protein, as shown in figure 29.

Finally, arguments were made against the search for a mathematical expression capable of predicting target protein (GFP) as an arabinose function. This is thanks to a graphic analysis of GFP versus arabinose concentration, indicating that dependence is quickly lost. Consequently, increasing arabinose concentration will raise that of GFP insignificantly. It seems that a minimum arabinose concentration yields a good GFP production. Nevertheless, it is possible that the time it takes for the system to reach equilibrium state its dependent con arabinose concentration. Experimentation for analyzing this time and the initial concentration of arabinose is needed to make any kind of correlation. All this ends in a simple equation predicting target protein concentration in equilibrium in function of biomass concentration. Graphic analysis also identifies there is a strong correlation between target protein in equilibrium and activator protein kinetic stability. The more kinetically stable the activator protein is, the more target protein is produced. However, if the protein is too stable it will tend to accumulate inside the chassis causing stress. A search for the optimal stability that yields the maximum target protein concentration obtainable and the minimal stress possible is suggested.

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Appendices

Experimental Protocol for Transformation

Estimated work time: 1 hour
Estimated total time: 3 hours (additional 14-18 hours for incubation)

Materials

Resuspended DNA	Resuspend the Wells of DNA with 10 µL of distilled water. With a pipette, press up and down several times. Let the mix rest for some minutes. The resuspended mix Will be red thanks to the dye cresol red.
10 pg/ul of control DNA	**1µl for the control transformation.** pSB1C3 w / *BBa_J04450*, RFP in the plasmid which is resistant to chloramphenicol and has a high number of copies.
Competent cells	**50 µl per transformation**. iGEM HQ stores competent cells in aliquots of 260 µl (5rxns in total) at -80 ° C.
Microtubes of 2 mL	**A tube per transformation**. Label the tubes with the name of the part or the location in the well before starting.
Floating grid made of foam for the tubes	Place the tubes of 2 mL in the grid for a better support while working with ice and for the thermic shock in the water bath.
Ice and an ice bucket	Fill the bucket with ice and pre-cool the tubes of 2mL for 5 minutes. Defrost the competent cells stock in ice for 10 to 15 minutes.
Timer	
Water bath at 42°C	Adjust the water bath at 42°C before starting.
SOC Medium	**200 µl per transformation.** SOC Medium is better than the LB Medium for a higher transformation yield. The SOC Mediums need to be free from antibiotics and can get easily contaminated.
Incubator at 37°C	Preferably with a rotor or an agitator for the 2mL tubes. Incubate the Petri plates

	overnight (without agitation).
Petri plates with LB agar and antibiotic	2 plates per transformation. For the 20 µl and 200 µl. Make sure to use the correct antibiotic. Label with the name of the part or the location of the well before starting.
Sterile spreader or glass beads	They are used to spread the transformation mix through the Petri plates. Make sure to use a sterile technique between the plates.
Pipettes and pipette tips	We recommend pipettes and pipette tips of 10µl, 20µl, 200µl.

Setup:

When transforming competent cells, both the timing and temperature have a huge impact in the process. Use a timer, keep track of the incubation temperature, and maintain the materials in ice when necessary.

Resuspend the DNA in the selected wells of the distribution kit. Label the tubes of 2mL with the name of the part or the location of the well. Fill the bucket with ice and pre-cool the 2mL tubes (1 tube per transformation, including the control) in a floating foam grid.

1. Defrost the competent cells in ice

This step can take between 10 and 15 minutes for the broth of 260 µl. Get rid of the competent cells not used. Do not re-frost the unused defrosted cells since this process will drastically reduce the transformation efficiency.

2. Take 50 µl of the competent cells in a 2 mL tube with a pipette.

50µl in a 2mL tube per transformation. The tubes must be labelled, pre-cooled and put in the grid for support. Keep all the tubes in ice. Do not forget the control tube.

3. Take 1 µl of resuspended DNA in a tube of 2 mL with a pipette.

Take from the well to the tube correctly labelled. Do it gently up and down sometimes. Keep all the tubes in ice.

4. Take 1 µl of the control DNA control in a 2 mL tube

Take 1 µl for control of 10 pg/µl for your control transformation. Do it gently up and down a few times. Keep all the tubes in ice.

5. Close the 2 mL tubes, incubate on ice for 30 minutes.

The tubes can be smoothly agitated to mix the solution, but they must be immediately placed in ice after.

6. Thermic shock for 1 minute.

The tubes of 2 mL must be in the grid. Place them in the water bath to ensure that they are completely submerged. Time is crucial.

7. Incubate in ice for 5 minutes.

Place again the transformation tubes in the bucket of ice.

8. Take 200 µl of LB Medium for each transformation.

The LB Medium must be stored at 4°C, but it can be heated to room temperature before using it. Check if there is any contamination.

10. Place each transformation in 2 Petri plates, using a pipette, to obtain one plate of 20 µl and one of 200 µl.

Place 20 µl and 200 µl of the transformation under the plates which should be correctly labelled. Spread it with a sterile spreader or glass beads immediately. This will guarantee that only one colony will be selected.

11. Incubate the transformations overnight (14-18h) at 37°C.

Incubate the plates upside down (with the side that contains the agar on the top). If you incubate for too long, the colonies may grow too much, and the antibiotics may start to decompose causing the untransformed cell to start growing.

12. Count the colonies for the transformation control.

Count the colonies in the control plate of 20 µl and estimate the efficiency of the competent cells. The competent cells must have an efficiency between 1,5 x 10^8 to 6 x 10^8 ufc / µg of DNA.

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Experimental protocol for plasmid extraction (Miniprep)

Materials

- Biopure plasmid extraction kit.
- Problem Sample
- 4 sterile tubes (1.5mL)
- Sterile micropipettes tips

Equipment

- Micropipettes set
- Microcentrifuge, variable speed preferably

Method

1.- Precipitate biomass in tubes to 15000rpm, 5min. Pour supernatant (medium located) into a waste collection container.

2.-Resuspend sedimented bacteria cells into 250uL Buffer 1(Kept at 4°C), then transfer to a microcentrifuge tube. Cell lumps should not be seen after resuspending sediments.

3.-Add 250uL of Buffer 2 and invert smoothly 4 or 6 times to mix up. Do not shake on the vortex. If needed, go on inverting the tube until the solution turns viscous and slightly filmy. Do not let more than 5 minutes pass from step 3 to 4.

4.-Add 350uL of Buffer 3 and mix up the tube by inverting smoothly 4 to 6 times right after adding the buffer. Note: Buffer 3 should be added into the lower part of the tube. Once the solution´s been inverted it should look murky.

5.-Centrifuge 10min, 13000rpm into a microcentrifuge. A white precipitate should form.

6.- Withdraw supernatant from step 4[... ambiguo]

7.-Centrifuge for 30 or 60s. Castaway supernatant.

8.- Add 750 uL of PE buffer to the centrifugation column, and centrifuge for 60s.

9.-Castaway constant flow and centrifuge for 1 minute.

10.- IMPORTANT: Washing waste buffer won't be completely eliminated unless the constant flow is casted away before the additional centrifugation. Residual ethanol from PE buffer may inhibit later enzymatic reactions.

11.- Set the column into a 1.5mL clean microcentrifuge tube. Add 50uL of EB Buffer or distilled water into the column center. Let the extraction rest for 1 minute and centrifuge once more for a minute without withdrawing the permeate.

Ollin SynBio

Experimental protocol for enzyme digestion and ligation

Estimated time: 30 min – 1 hour

Before start: Keep all reagents and tubes in ice.

Materials

- 8 tubes strip or (3) 0.6mL thin-walled tubes.
- Biobrick plasmid (DNA purified >16ng/uL)
- Molecular biology grade distilled water.
- BSA (Fetal Bovine Serum)
- Restriction Enzymes: EcoRI, SpeI, XbaI and PstI

Reaction Buffer

- 10mM Tris-HCl
- 10mM MgCl2
- 100ug/mL BSA
- pH 7.9, 25°C
- 50 mM NaCl

Equipment

- Ice and container
- Thermal cycler or thermoblock

Methodology

1.- Add 250ng of DNA to digest and adjust the volume with distilled water.

2.- Add 2.5 uL of Reaction Buffer.

3.- Add 0.5uL of BSA.

4.-Add 0.5uL of EcoRI

5.-Add 0.5uL of PstI

6.- A total volume amount of 20uL should be in the tube. Mingle thoroughly and give a soft centrifuge spin.

7.-Incubate the digestion mix set to 24°C- 25°C for 30min, then set to 80°C for 20min to disable the enzymatic reaction. Incubate in a thermocycler or thermoblock.

8.-After the incubation is done, run a portion from the digestion mix into a gel (8uL, 100ng) to bear out whether the plasmid as well as the insert have the expected length.

9.-Add 2uL of digested plasmid (25ng).

10.- Add an equimolar portion from digested fragment of: EcoRI, SpeI (<3uL) *

11.-Add an equimolar portion from digested fragments of: XbaI, PstI (<3uL) *.

12.-Add 1uL of T4 DNA ligase buffer. Do not use rapid ligase.

13.-Add 0.5uL of T4 DNA ligase.

14.-Add water to reach a total volume of 10uL.

15.-Undergo ligation, 16°C, 30min, then disable enzymatic reaction to 80°C, 20min.

16.-Transform with 1-2uL from the product.

***Note: Incubation temperatures set are considered as restriction enzymes used are wildtype.**

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Experimental protocol for electrophoresis in agarose gel

Materials

- TAE (recipe at the end of the protocol)
- Agarose
- Intercalating reagent

Equipment

- Casting tray
- Well combs
- Voltage source
- Gel box
- UV light source
- Microwave

Methodology

Pouring a Standard 1% Agarose Gel:

1. Measure 1 g of agarose.
2. Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
3. Microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up). You can be sure that the agarose is completely dissolved when the solution turns crystalline.
4. Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.

5. *(Optional)* Add your preferred intercalating reagent to the recommended concentration (For more information review the SS of your preferred product). The intercalating reagent binds to the DNA, allowing you to visualize the DNA under UV or blue light.
6. Pour the agarose into a gel tray with the well comb in place.
7. Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

Loading Samples and Running an Agarose Gel:

1. Add up to 2-4uL of your preferred loading dye to each one of your DNA samples and mix it using the micropipette.
2. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
3. Fill the gel box with 1xTAE until the gel is covered.
4. Carefully load a molecular weight ladder into the first lane of the gel.
5. Carefully load your samples into the additional wells of the gel. Use a new micropipette tip to load each one of your samples.
6. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.

   Note: Be careful that your DNA does not run out of your agarose gel.

7. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
8. Using any device that has UV or blue light, visualize your DNA fragments. The fragments of DNA are usually referred to as 'bands' due to their appearance on the gel.

Recipe for TAE
One liter 50X stock of TAE

- Tris-base: 242 g
- Acetate (100% acetic acid): 57.1 ml
- EDTA: 100 ml 0.5M sodium EDTA
- Add dH2O up to one litre

Important: To make 1x TAE from 50X TAE stock, dilute 20ml of stock into 980 ml of DI water

Ollin SynBio

Experimental Protocol for standard PCR (Polymerase chain-reaction)

Estimated time: 2 - 3 hr depending on the number of cycles

Materials

Quantity	Component	Final concentration
24.3 µL	PCR-grade water	
5 µL	PCR buffer 10x	1x
1 µL	dNTP Mix	200 µM
8.75 µL	Forward primer	0.1-0.5 µM
8.75 µL	Reverse primer	0.1-0.5 µM
0.5 µL	*Taq* DNA polymerase*	0.05 units/µL
1uL	DNA sample	200 pg/µL
0.7 µL	25 mM MgCl2	0.1-0.5 mM
50 µL	Final reaction volume	

**Thermus aquaticus "Taq"* DNA polymerase, without $MgCl_2$

Equipment

- Thermocycler or 3 boiling water baths
- 3 thermometers
- Eppendorf tubes or microtubes
- Floaters

Method:

1. Add the reagents on an Eppendorf tube or microtube in the order as they are listed in table 1. materials. For a big number of reactions, a master mix should be prepared with the DNA sample and aliquots of the mix are taken and placed in tubes for reaction, finally, DNA sample is added to the aliquoted tubes.
2. Shake the tubes with the PCR mix softly around 27 times in a minute with the arm at a 75° angle to assure the homogeneity of the mix.
3. Centrifugate briefly to gather all the components at the bottom of the tube.
4. Add 50 µL of mineral oil to the top half of each tube if a thermocycler without a hot lid is used in order to avoid evaporation
5. If you have a thermocycler, perform the reaction with the parameters established in the following Table.

Table 2. Typical cycle parameters.
25–30 cycles are recommended for amplification.

PCR stage	Temperature (°C)	Duration
Denaturation	94 °C	1 min
Annealing	55 °C	2 min
Extension	72 °C	3 min

If you do not have a thermocycler, you can perform the reaction using three boiling water baths or heat blocks. Each one must be kept at a temperature interval shown in the following table.

Tabla 3. Cycle parameters for PCr without a thermocycler.

35–40 cycles are recommended for amplification.

PCR stage	Temperature (°C)	Duration
Denaturation	90 °C – 100 °C	1 min
Annealing	50 °C – 60 °C	2 min
Extension	70 °C – 80 °C	3 min

Note: It is important to constantly monitor and control the temperature in each stage, seeking that the temperature is always under the interval.

6. If you work with water baths it is important to have floaters to place the PCR tubes.
7. The tubes will first be placed on the denaturation container. The tubes will be changed from container to container and they must be left in each container at the specified time in table 3. Each cycle starts with denaturation, followed by annealing and lastly, extension. It is VITAL that this sequence is followed for the PCR to function.

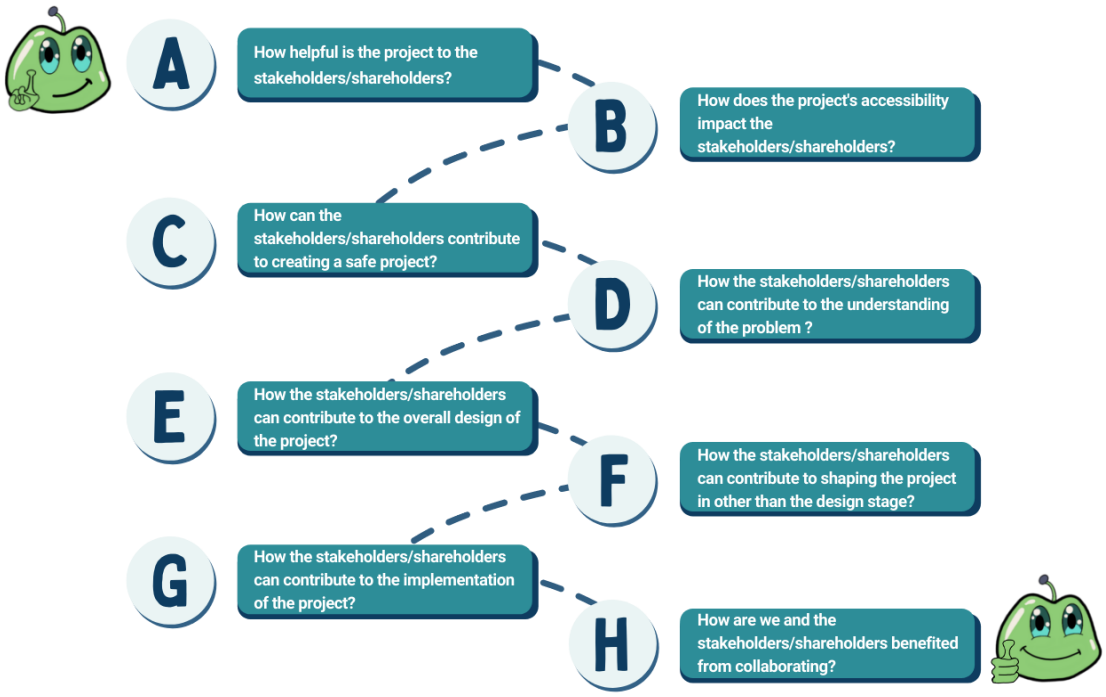
   Denaturation > Annealing > Extension
8. Once the cycles are done, let the tubes cool down in a container with ice water.
9. The amplified DNA can be evaluated by an agarose gel electrophoresis with ethidium bromide staining (or other nucleic acid stain, f.e. SybrGreen)
   Note: The mineral oil layer can be eliminated by using an extraction with chloroform (1:1), and retrieving the aqueous phase.

Social Impact

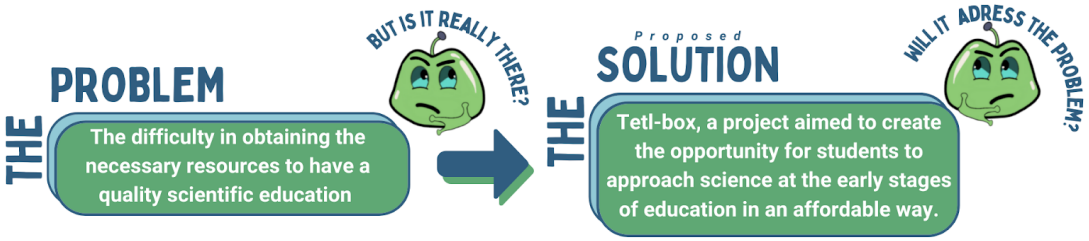
To make sure our project would have a positive impact in our community, we first differentiated and identified the stakeholders and the shareholders. The team defines the stakeholders as those who impact or are impacted by the team's objectives. For example, students and teachers, given that they are potential users of Tetl-Box and can impact the team's achievement of the established goals. On the other hand, the shareholders have the authority to decide which resources are bought and used. For example, even though a teacher would like to implement Tetl-Box, he may not have the power to determine if the school resources should be invested. This responsibility usually lies within the administration faculties.

We classified the stakeholders using a value matrix to determine how they could impact the project's development.

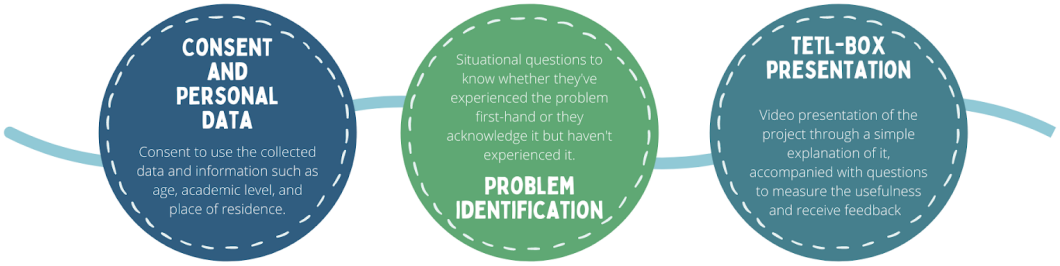
The classification was done based on 8 factors that represent the different ways the stakeholders and shareholders can help shape our project.



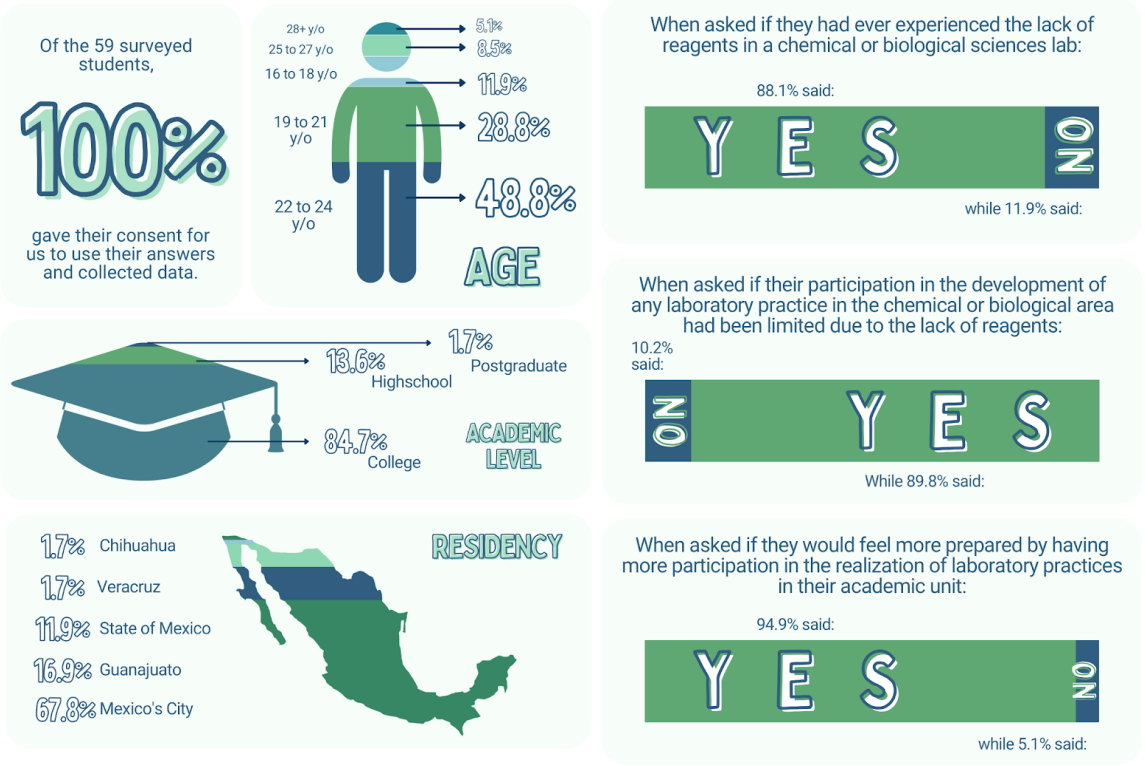
So, to measure the impact of it, we first had to understand the problem we were tackling and find out if our proposed solution was a way to address it.



To accomplish that, we created a survey addressed to highschool and college students, where we included questions to find out if they perceived that situation as a real problem. The survey was divided in three sections:



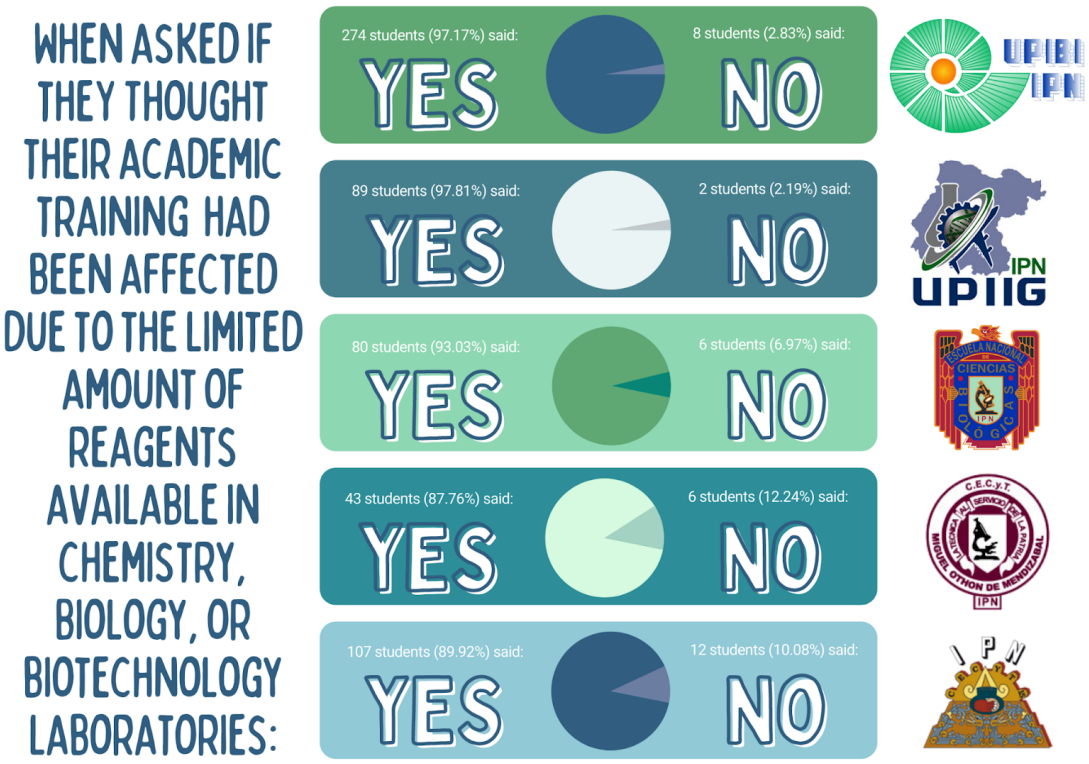
The results obtained from the first two sections are presented below:



These indicate the existence of the problem and how it is recognized by students from distinct backgrounds. The results from section 3 are shown in the proof of concept, which is where the solution part is approached.

It is important to mention that we designed this survey methodologically, taking into account different resources and the expertise from one of our mentors, Ph.D. Navarrete. We wanted the surveys to be as clear as possible for the students and not bias or somehow influence their answers, so we could collect objective data.

We also conducted quick polls through social media, specifically in highschool and college faculties' Facebook groups, the results are shown below.



Although these polls were simpler than the survey, we can see the results coincide.

Analysing the problem made us understand that although our project was already tackling some of the causes of the scientific education accessibility problems in LATAM, there are other aspects that we need to consider. So to contribute to the solution from different angles, we also implemented actions beyond Tetl-Box. Which are presented below:

Diverse perspectives analysis

"Diverse perspectives analysis" was designed to **understand the problem from the point of view of people affected by it**, who find themselves in different social and educational situations. To make their problems visible and propose possible solutions. We used the interview as a method of qualitative analysis. Ph.D. Navarrete, one of our team's mentors, guided us through the entire process for this activity, from profile selection and interview structure to the analysis of the results.

First, we defined a **variety of profiles** of people that could be impacted by Tetl-Box and represented a social group. Then we looked for stakeholders that could fit that profile or had that background. These profiles are listed underneath, and the people we reached out to, covered one or more of these :

- Long time researcher.
- Researcher with any visual, hearing, or language impairment.
- Researcher from an indigenous community.
- Students with any visual, physical, hearing, or language impairment.

- Student from an indigenous community.
- Students or researchers from different regions of the republic.
- Researcher of a private agency.
- Students from underserved areas.

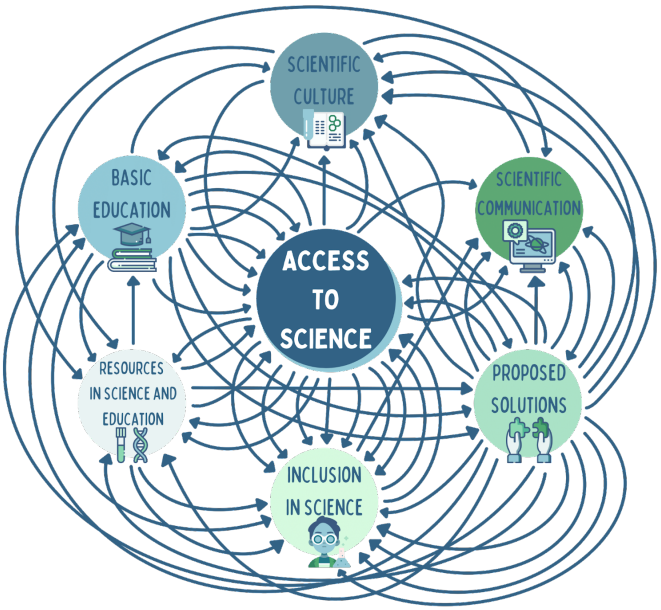
Then we conducted a **group interview**, so people were able to listen to different experiences and points of view and enrich their own. They shared the way they perceive **four main topics**:

- Access to science and technology educational resources.
- Disparity and difficulty of access to these resources.
- Importance of promoting science and technology.
- Difficulty developing in the scientific field.

Fragments of "Diverse perspectives analysis interview", conducted by Ollin SynBio, September, 15th, 2021. Click on the image to watch the video.

The results are shown in the figure below. It can be noticed that the main problem discussed is the **difficulty to access science in Mexico.** The circles around the main problem represent six more categories related to it. The arrows indicate how a category impacts another. Each arrow symbolizes a different highlighted issue. Some of the issues our stakeholders mentioned were: **lack of resources in education, gender gap, need for inclusion in science, need for an update in academic programs,** and **scarcity of opportunities to develop in STEM**, among others.

Possible solutions emerged too. Interviewees agreed on: **professionalization of science communication, public policies to promote scientific education, additional investment in elementary education,** and more.



There was consent that **providing accessible resources would help alleviate some of the existing problems around access to science in Mexico**. Scientific communication and improving our educational system are as important.

From this analysis, we can also note the urgent need for **inclusive science.** We developed some activities to make this problem visible.

And how does our project contribute to the solution?

We presented Tetl-Box to the interviewees through a video and asked for their **feedback and concerns** according to their backgrounds and expertise. The following 3 points stand out from their responses:

The proposed experiments need to be all related, they should tell a story. And it needs to be well-equilibrated so it doesn't generate dangerous waste.

Think about how to adapt the project to include people with any visual, physical, hearing, or language imparities.

Consider also offering low-cost equipment to conduct Tetl-Box proposed experiments.

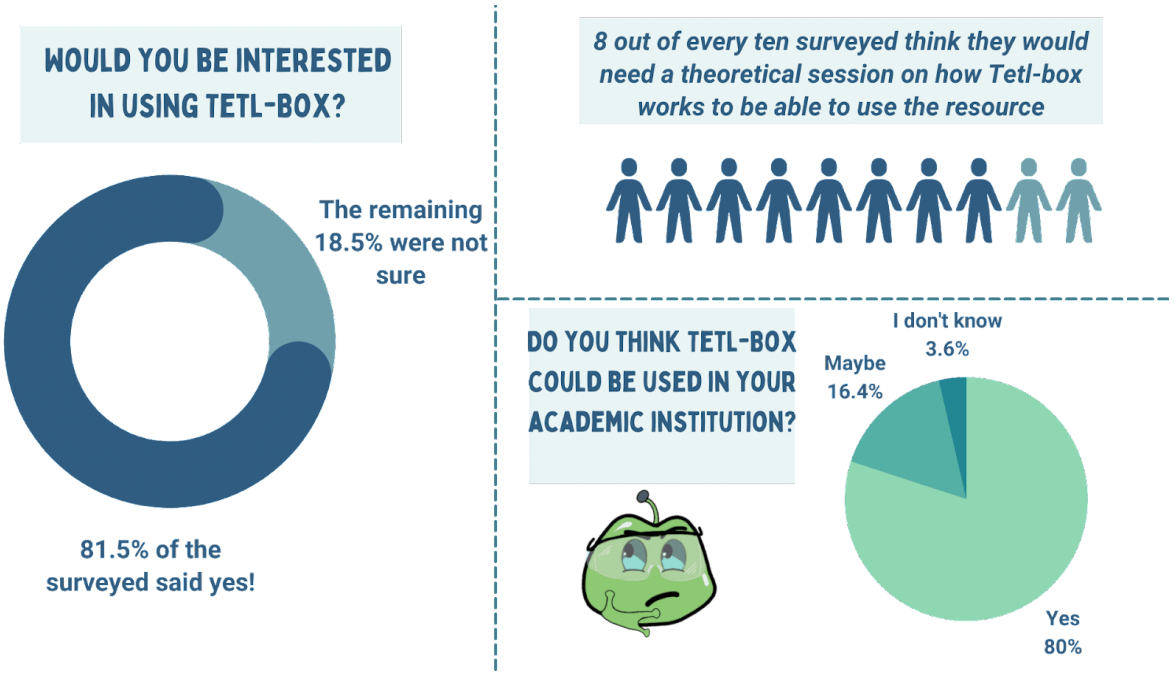
The first two were considered in the project's design: in the **Risk Assessment** and in our **actions to promote inclusion in science.** The third is something we have contemplated from the beginning of the project. For now, we decided to focus our energy and resources on reagent accessibility. Nonetheless, we are working on creating **strategic alliances** with those who are addressing this particular matter.
Our project can contribute to the solution for the problem of access to science in Mexico. But, it must be remarked that this is not the only action. Significant **changes are required in society and strategies** need to be implemented related to government policies and the education system.

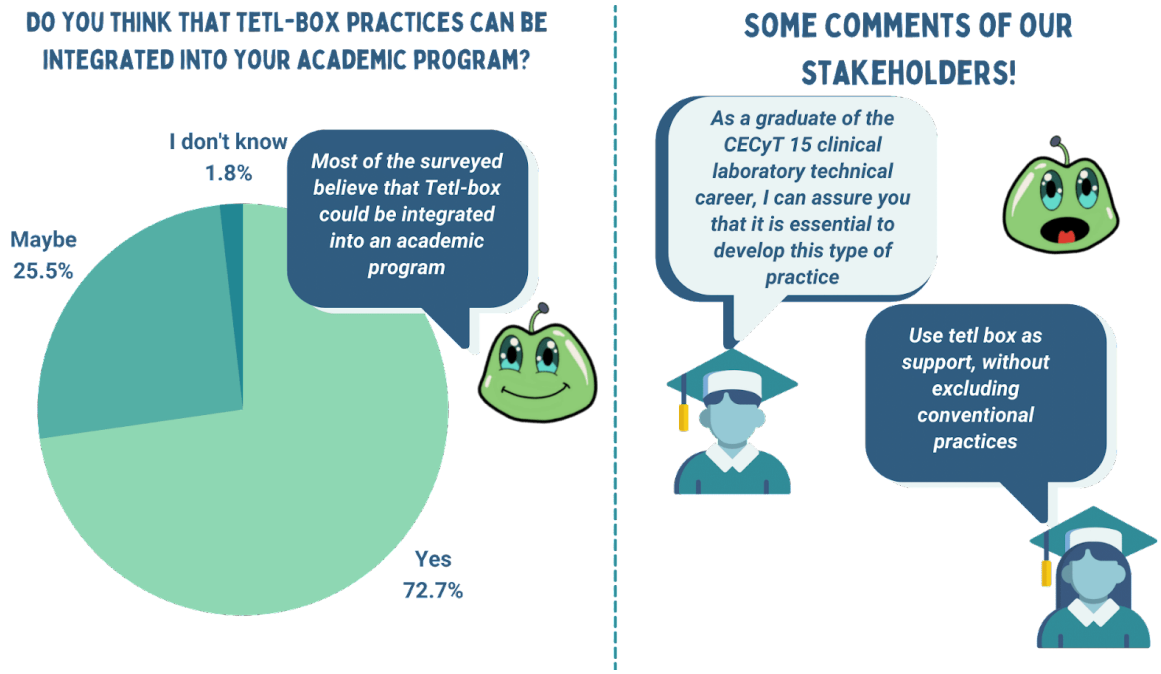
Proof of Concept

We wanted to make sure that our project works for the stakeholders, not just on paper or according to our standards. For that reason, we decided to do a proof of concept. For this, we developed a survey to collect opinions about our project from the target group (high school and undergrad students). Before they expressed their opinion, we presented a video explaining what Tetl-Box is in simple terms. You can watch the video right below.

The stakeholders also interacted with our deliverables to prove if they helped accomplish the objectives for which they are intended.

Among our results, we can highlight the ones shown below:





We also developed procedures for activities targeted to high school and college teachers:

The first one consisted of a comparative test for the protocols developed, where we also provided standard protocols for the same experiments to receive comments and recommendations based on the teacher's experience. The standard protocols are meant to give the teachers a starting point for their analysis, given that in high school, these practices are not always taught.

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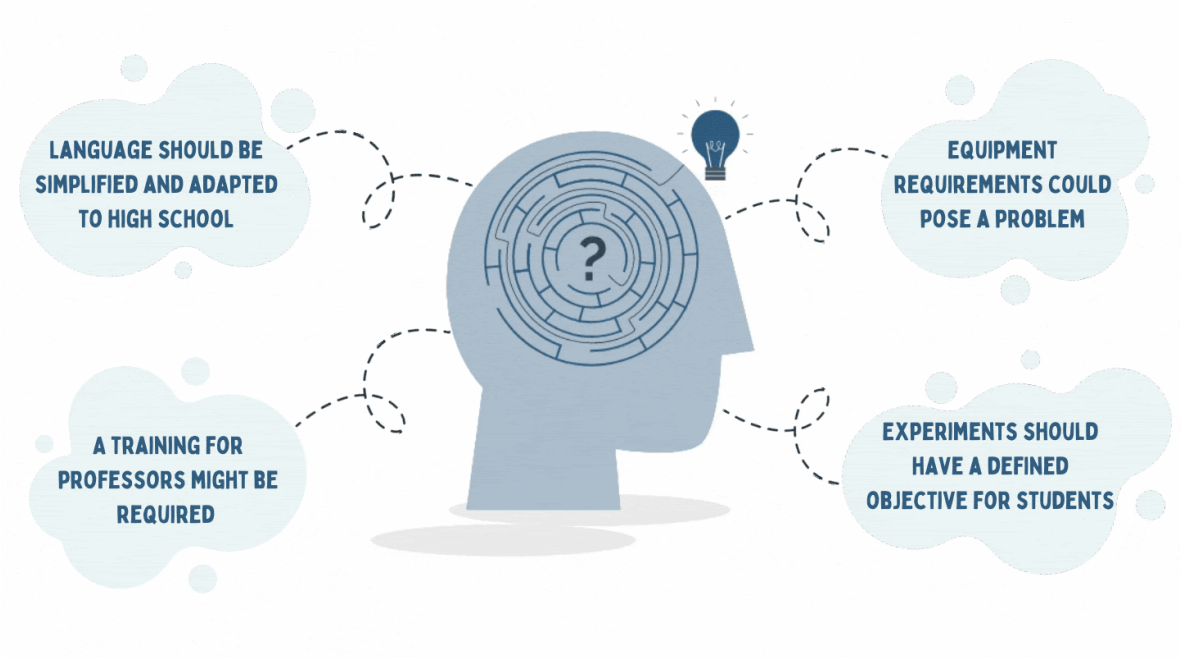
From the first activity, we rescued four ideas. We will implement modifications in our project to improve the clarity and simplicity of the protocols. So high school students can have a better understanding of the fundamentals of the experiment. While also clarifying and defining the purpose of the project so students can have a concrete objective. On the other hand, we will continue to find strategic alliances that allow the team to ensure equipment won't be a barrier.



The second will be a set of practice drills, with teachers explaining the protocols to students.

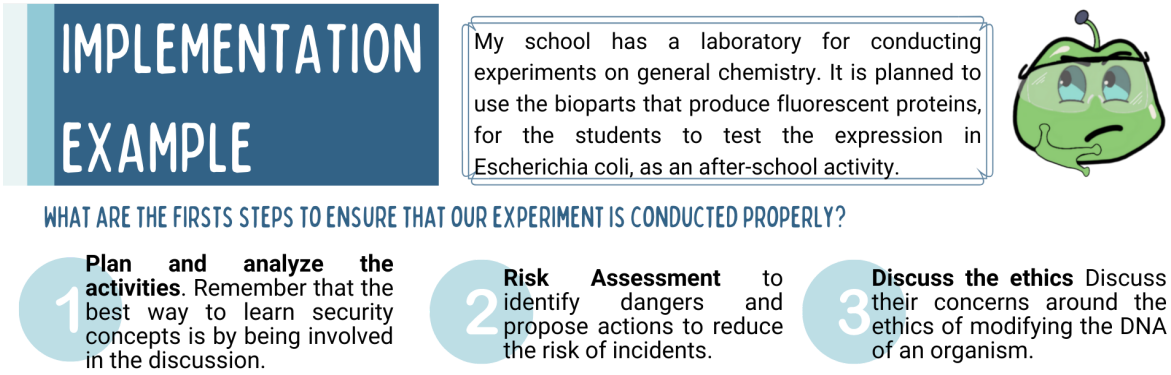
Biosafety Considerations

“Bioethical Principles and Biosafety Guidelines”

One of the actions implemented to ensure Tetl-Box is used safely and responsibly was the establishment of “Bioethical Principles and Biosafety Guidelines”. With the risk assessment, we found that, among other things, we could warn the users about the hazards that may occur during the use of this tool, and indicate how to prevent

them. Biosafety User Guidelines were developed around four main topics: general safety, workspace, user protection/safety, and waste management.
Ethics allow us to act with respect, benevolence, and honesty to minimize risks to the user and his surroundings. That is why, eight Bioethical Principles were included, addressing respect for life, promotion of education, and commitment to biosafety.

Having in mind Tetl-Box pursues education accessibility, an implementation example was included. With this, users can adapt spaces to conduct experiments with Tetl-Box and its products as long as they identify the risks and propose solutions to avoid or reduce them. This implementation example helps to understand the topics described in the document too. We also encourage a deep discussion, which consists of an activity that stems from a simple question: Why do we want to conduct the experiment? In this activity, it is proposed students share their concerns about performing a genetic modification while discussing whether these are justified.

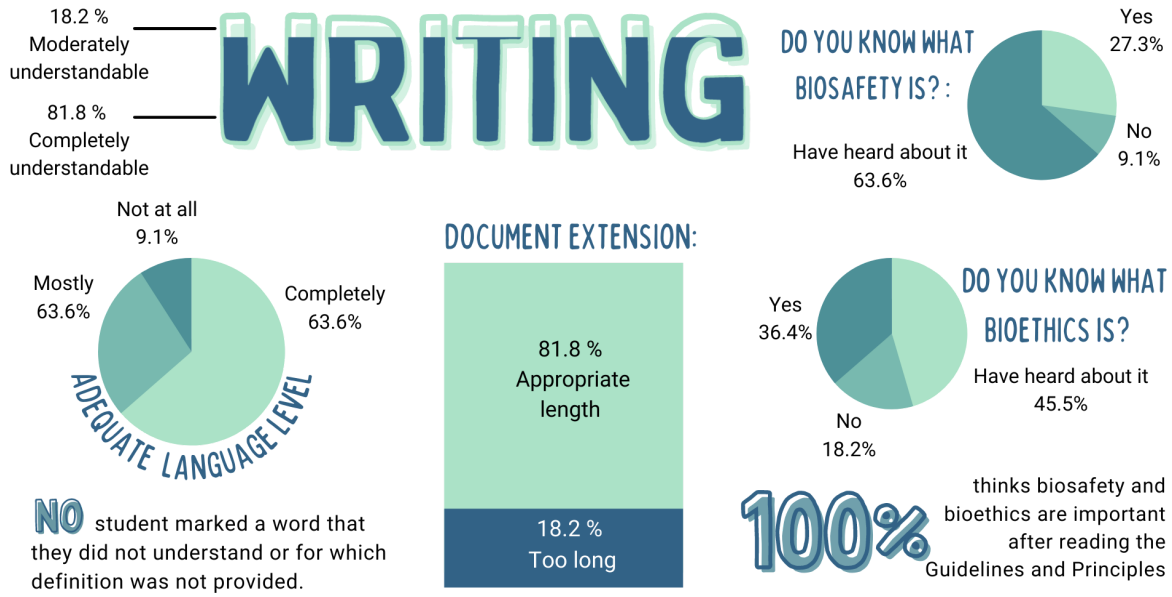


Finally, the document incorporates a Regulatory Affairs Section and recommended bibliography depending on the application the user attempts to give to Tetl-Box.

But, would this resource be helpful? Would it be understandable?

We presented Tetl-Box in a short video and asked high school students to read the “Bioethical Principles and Biosafety Guidelines” and asked their opinion related to the content and whether it would help minimize risks.

These were the results:



The need to provide this resource, which appeared from the risk assessment, was confirmed. All students agreed that applying the Bioethics and Biosafety recommendations may help ensure theirs and the environment's safety while using Tetl-Box. They also shared feedback and suggestions.

> "Try to do a shorter version students can easily use while conducting the experiments with graphics that enlist all the recommendations"
>
> *-5th-semester high school student from Chihuahua*

Please review the support document in the next link:
Bioethical_Principles_and_guidelines.pdf

Assessment Group:

In Ollin SynBio, we are strongly committed to the development of projects that are safe for the user, for the environment, and have bioethical integrity. So, to ensure the safety and integrity of our project, we called for the formation of an Assessment Group made up of experts in the Biosecurity/Biosafety/Bioethics field and-or people trained in Bioethics and Biosafety.

The central duties of the expert group were to assess the biosafety and biosecurity actions implemented and deliver recommendations to ensure the development of a safe project.

Meet the experts who participated in this group!

JOSÉ A. ALONSO

Ph. D. Social Studies of Science (UNAM)
M. in Bioethics (University of Pennsylvania)
B. Sc. in Genomics

CYNTHIA G. SÁMANO

Ph. D. Neurobiology (SISSA)
D. Sc. Biomedics (UNAM)
B.Sc. Biology (UNAM)

ALBERTO E. LÓPEZ

M. Sc. Environmental Management (UNFV)
Biologist with a mention in Cellular and Genetic Biology (UNMSM).
B. in education (UPCH)

Diversity and inclusion

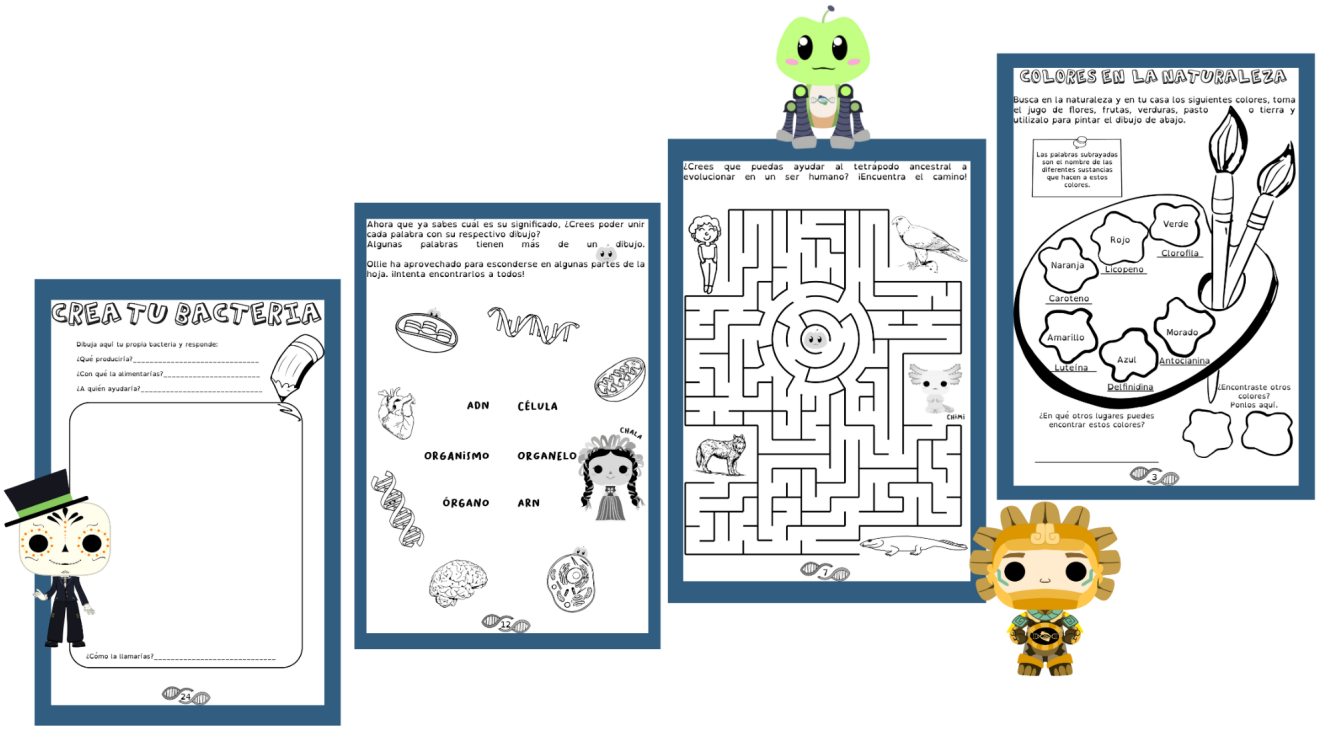
As it was mentioned before, science in Mexico and Latam is often expensive and very hard to access for everyone. The high cost related to scientific project development represents a barrier for achieving progress and improvement of science.

In Mexico, vulnerable groups await even more social and learning barriers to get access to science and technology, also adding the economic barrier and the discrimination it brings with it. Resulting in less people interested in studying a STEM related career.

To get rid of these inequalities, we came up with the idea of Ollibro, which is a tool that aims to introduce and encourage interest in science around the children who read and interact with it. Their age will determine the type, amount, and form of the information so that they can take full advantage of it and generate curiosity.

Initially, Ollibro intended to be a storybook describing the histories, legends, and myths lived by Ollie about the presence and importance of science in every aspect of our lives. Nonetheless, that was not the best way of introducing and presenting the scientific world to children.

We had to surpass these obstacles and come with a proposal capable of fulfilling diffusion and interest in science in kids. A lot of redesigns were involved in the materialization of this new book. Consultation with stakeholders and team members was necessary, so they could provide feedback on what was good and what needed a change.



When analyzing the data that different sources presented to us, it was found that Chiapas is the poorest state in the country (Figure X), as well as the second entity with the highest number of speakers of indigenous languages (Figure Y), in this case being Tseltal the main one (Table X).

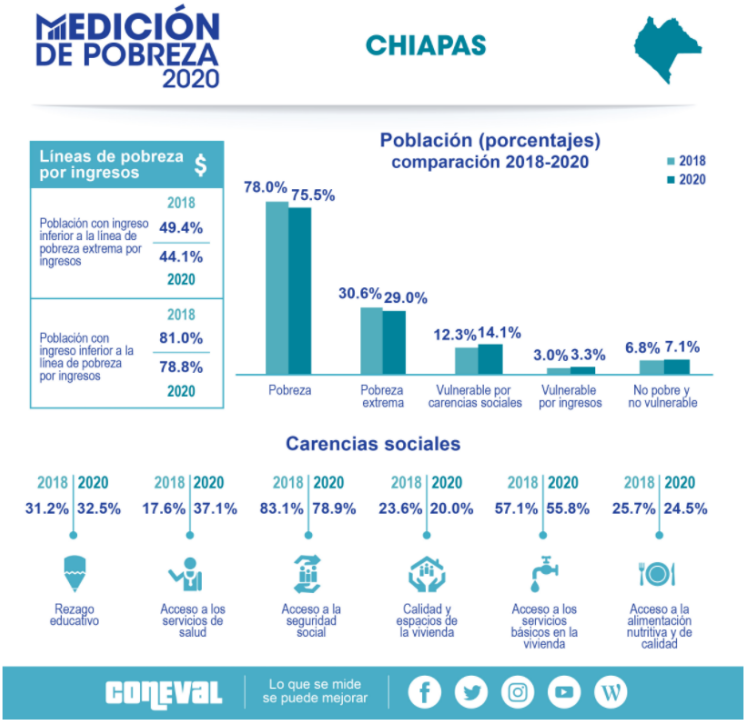


Figure X. *General results of poverty measurement in the state of Chiapas 2020. Source: Estadísticas de pobreza en Chiapas, CONEVAL*

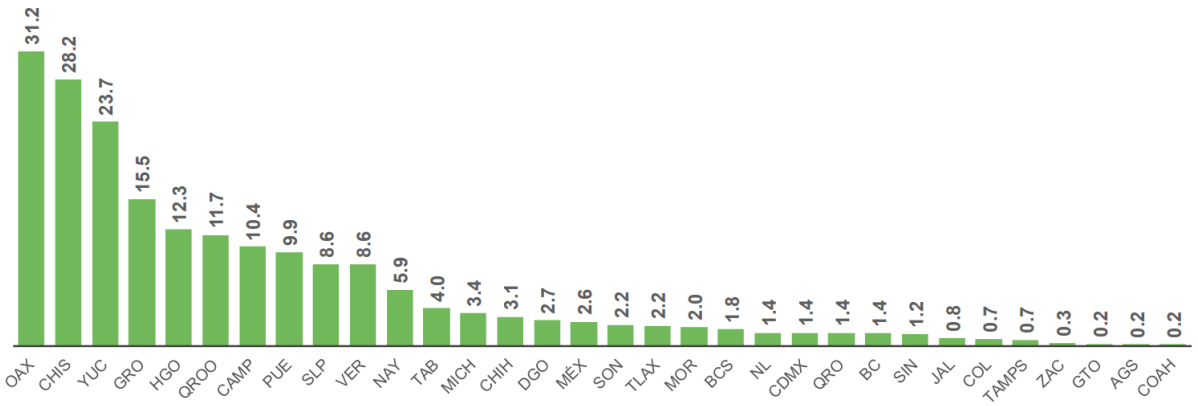


Figure Y. Population percentage of 3 or more years speaker of an indigenous language per state in Mexico.
Source: Censo de Población y vivienda 2020, INEGI.

***Table X**. Main indigenous languages spoken by population with 3 or more years by district in Chiapas.*
Source: Programa educativo estatal de la población indígena 2018 - Chiapas, INEE.

Lengua indígena hablada	Total estatal		Región																	
			Centro		Altos		Fronteriza		Frailesca		Norte		Selva		Sierra		Soconusco		Istmo-Costa	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Tseltal	537 477	39.5	13 778	13.9	152 647	33.8	6 036	8.5	3 411	28.8	7 076	4.7	354 310	62.6	15	0.6	144	1.9	60	6.3
Tsotsil	473 491	34.8	60 344	60.8	287 586	63.6	7 077	9.9	7 834	66.1	104 605	69.9	5 279	0.9	39	1.5	401	5.4	326	34.4
Ch'ol	210 058	15.4	1 783	1.8	1 017	0.2	1 176	1.7	3	0.0	4 232	2.8	201 799	35.6	5	0.2	37	0.5	6	0.6
Zoque	55 529	4.1	20 503	20.7	113	0.0	10	0.0	46	0.4	33 132	22.2	1 700	0.3	4	0.2	19	0.3	2	0.2
Tojolabal	54 686	4.0	61	0.1	9 977	2.2	44 434	62.3	12	0.1	7	0.0	164	0.0	8	0.3	23	0.3	0	0.0
Mam	8 060	0.6	53	0.1	36	0.0	1 087	1.5	198	1.7	6	0.0	42	0.0	1 835	72.1	4 791	64.2	12	1.3
Zapoteco	2 335	0.2	1 024	1.0	50	0.0	57	0.1	21	0.2	62	0.0	77	0.0	11	0.4	649	8.7	384	40.5
Otros	19 613	1.4	1 735	1.7	692	0.2	11 389	16.0	318	2.7	460	0.3	2 838	0.5	627	24.6	1 396	18.7	158	16.7
Total	**1 361 249**	**100.0**	**99 281**	**100.0**	**452 118**	**100.0**	**71 266**	**100.0**	**11 843**	**100.0**	**149 580**	**100.0**	**566 209**	**100.0**	**2 544**	**100.0**	**7 460**	**100.0**	**948**	**100.0**

Coeficiente de variación ajustado mayor a 10%. Ver la nota técnica "Criterio de precisión" en *Panorama Educativo de México 2017* (INEE, 2018c, p. 171).
Fuente: cálculos con base en la Encuesta Intercensal 2015, INEGI (2015).

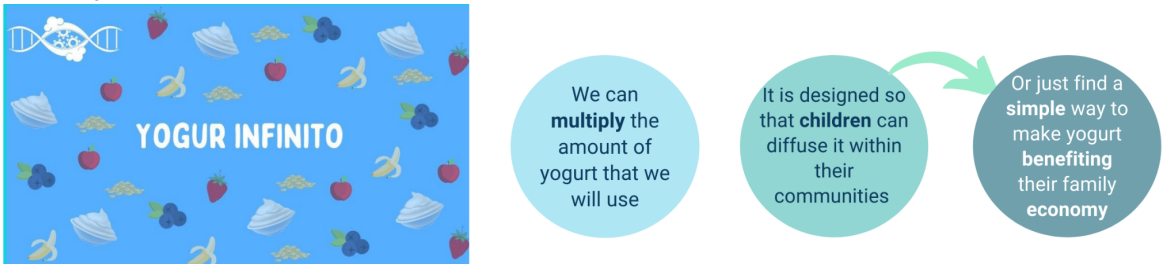
With the objective of making the biological sciences known in native communities, we selected the Tseltal language to make the translations of the resources generated by the team, as it is shown in the next image:

To prove the efficiency and effectiveness these interactive and dynamic activities had in teaching the concepts and subjects included in Ollibro, we prepared an application protocol with the help of some education expert stakeholders.

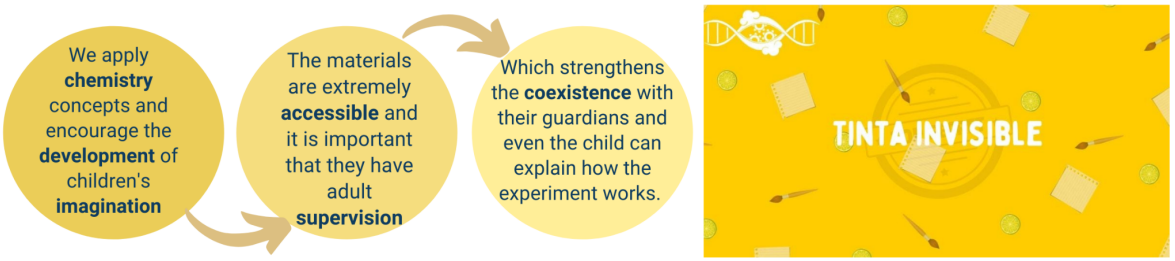
The protocol incorporates a detailed description of activities to evaluate the different interactive elements of Ollibro and their effectiveness in teaching. It will be helpful to test the Ollibro and see its acceptance in the targeted age.

To complement what we learned in the didactic book, we decided to make 3 videos of experiments. We chose the main topics related to science, which we knew would help to understand what it was about and above all to know the endless applications it has. The chosen experiments are:

Yogurt elaboration
https://youtu.be/aP6eLN2UMiA



Invisible Ink
https://youtu.be/MIvC5dVmhcY



DNA Extraction from a strawberry
https://youtu.be/k6OlRWOvTmk

Another consideration in choosing these experiments was to verify that they were low cost and did not require a lot of materials, so that they could be applied in a classroom or could be easily done at home. These videos are available on our Youtube channel and are subtitled so that people with hearing loss can watch them without problems.

The main objective of these video experiments and the Ollibro, is that children practice what they learn and have fun with that knowledge, making them more curious about science; another main objective is that, by requiring the supervision of an adult, this person will also be involved with what the child is learning, also awakening their interest in science.

References

*Consejo Nacional de Evaluación de la Política de Desarrollo Social. (2020). *Estadísticas de pobreza en Chiapas*. Retrieved from CONEVAL: https://www.coneval.org.mx/coordinacion/entidades/Chiapas/Paginas/principal.aspx

*Instituto Nacional de Estadística y Geografía. (2020). *Presentación de Resultados - Censo de población y vivienda 2020 - Estados Unidos Mexicanos*. Retrieved from INEGI: https://www.inegi.org.mx/contenidos/programas/ccpv/2020/doc/Censo2020_Principales_resultados_EUM.pdf

*Instituto Nacional para la Evaluación de la Educación. (2019). *Panorama educativo estatal de la población indígena 2018 - Chiapas*. Retrieved from INEE: https://www.inee.edu.mx/wp-content/uploads/2019/08/P3B113.pdf

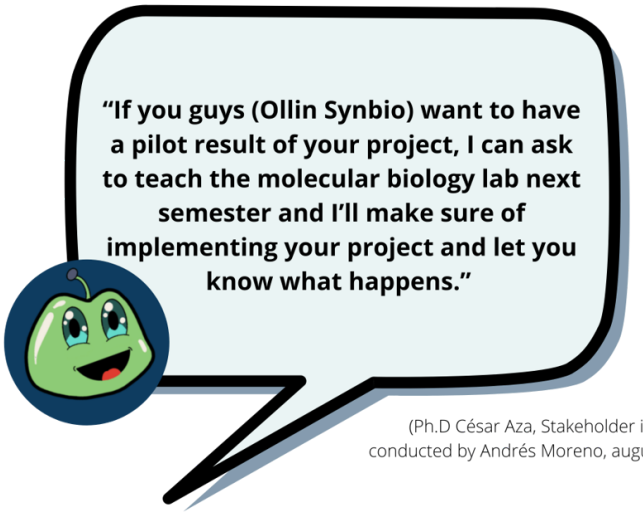
Finance and funding

Brief introduction to entrepreneurship and innovation

In Ollin SynBio we strongly believe that in order for our project to have the impact we are looking for and have a real life shot at making a change with synthetic biology, the project we are elaborating should solve a real problem and have real users. This is why we focused on creating an entrepreneurial vision for our project. At first it was difficult to pick a business model and visualize how this project could reach a real user. But little by little, we built a business and investment case with the use of tools and help from our mentors.

Customer discovery

We conducted several interviews with potential users and clients of our Tetl-box. These being university professors and researchers. Overall our potential users were interested and eager to try our product if we could reach price competitiveness and once it was out on the market. We figured professors and researchers could play as clients and users because they can be in liberty of buying their own reagents for their own use. We leave you paraphrasing the words of a teacher and an interview with the bio-entrepreneur María Pía Campot from Enteria who gave us valuable feedback about the market and potential of our project. (The latter document is not translated to English in order to maintain originality).



(Ph.D César Aza, Stakeholder interview, conducted by Andrés Moreno, august 16th 2021)

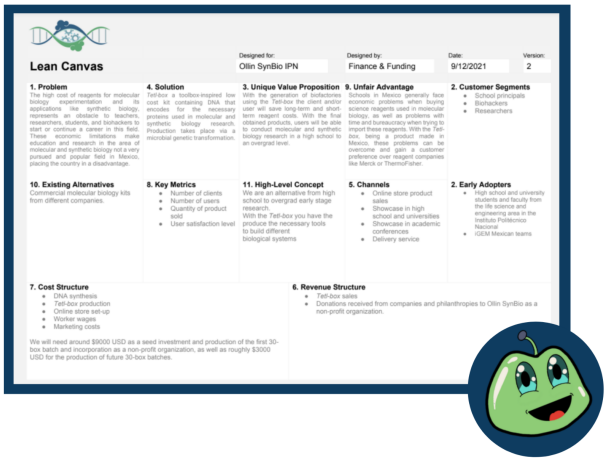
https://www.youtube.com/watch?v=jq4G9mmNhIs

Competitor analysis

We don't view ourselves as competitors but more as an alternative, since other reagent companies offer high purity and a longer list of hard-to-get reagents. We compared ourselves to Merck, ThermoFisher, and New England Biolabs. We found that these companies don't offer all of the reagents you can produce with the Tetl-box, but if you were to make your shopping list with these companies to buy almost all of the reagents we offer, it would cost you $1291 USD with Merck, $717 USD with ThermoFisher, and $1675 USD with New England Biolabs. With the Tetl-box, you can produce your own reagents for $250 USD. It is worth noting that some of these reagents are not available within one single provider, which implies the customer must search with other companies to obtain the same list of reagents we are offering.

Product	Merck	Thermofisher	New England Biolabs
Ligase	USD $89(100u)	USD $76(10u)	USD $65(20,000u)
Pfu Polymerase	Not available	USD $194(100u)	Not available
Invertase	USD $70(250mg)	Not available	Not available
XbaI	USD $41(1,000u)	USD $37(1,500u)	USD $72(3,000u)
Peroxidase	USD $64(1,000u)	USD $122(10mg)	Not available
Bst Polymerase	Not available	Not available	USD $72(500mg)
PstI	Not available	USD $32(3,000u)	USD $67(10,000u)
NotI	USD $438(1,000u)	USD $81(300u)	USD $75(500u)
Taq polymerase	USD $156(250u)	USD $175(500u)	USD $1249(20,000u)
SpeI	USD $433(1,000u)	Not available	USD $75(500u)

Lean Business Model Canvas:

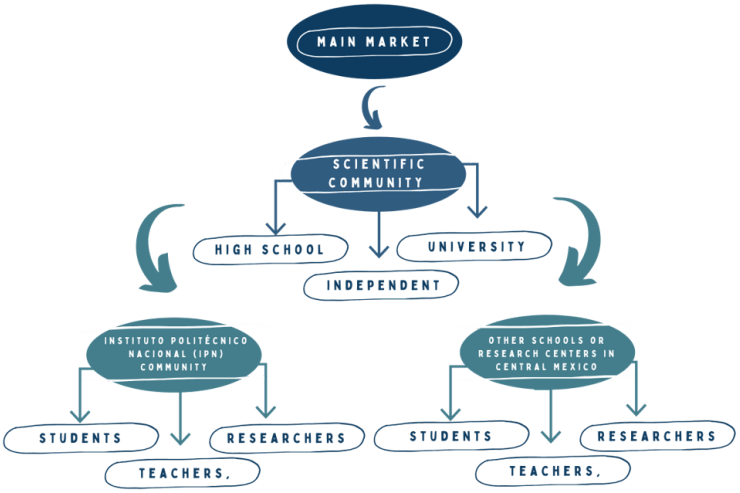


The tool the team chose to use to quickly assess our **business model, clients, users, unique value proposition**, and other useful information necessary to elaborate an entrepreneurial vision of the Tetl-box was the **Lean Business Model Canvas**.

Gantt and OKR

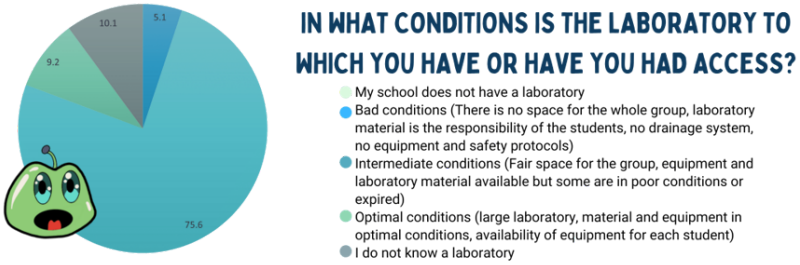
We defined our objectives, key metrics, and milestones, with the aim of planning the business model and determining its viability, as well as determining the budget for our product. Refer to the Gantt OKR document for more detailed information.

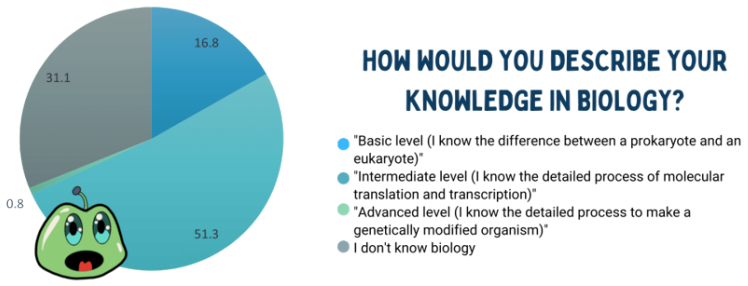
Market analysis:



Our market consists of **students, teachers, and researchers** mainly from the Instituto Politécnico Nacional (IPN) and other schools or research centers in central Mexico. Our school has a scientific force of 1,279 specialists recognized by the National System of Researchers (SNI). In addition to that, the IPN has 19 schools at the high school level, of which 5 of these are focused on the Biological Sciences area, and at the college level, there are 5 schools in the Medical Biological Sciences area and many other schools related to Engineering and Physical Mathematical Sciences. We would have a target user base of 37,215 students. Moreover, according to INEE at the national level, it is observed that **only 2% of the schools** in the country report having sufficient experimental science materials. This means there is plenty of opportunity to give molecular biology education to many schools.

We carried out an analysis of the conditions in which our key population is found, which was based on a field investigation, conducting surveys that will provide us with this information. In this analysis, we were able to observe and confirm that most of them are in intermediate conditions, they know laboratory material, but not all have used it or have ever heard about synthetic biology. **This tells us that our product covers a large part of the needs of students**, such as making a more accessible approach to science, expanding the scientific community, and providing the tools for reagent production.



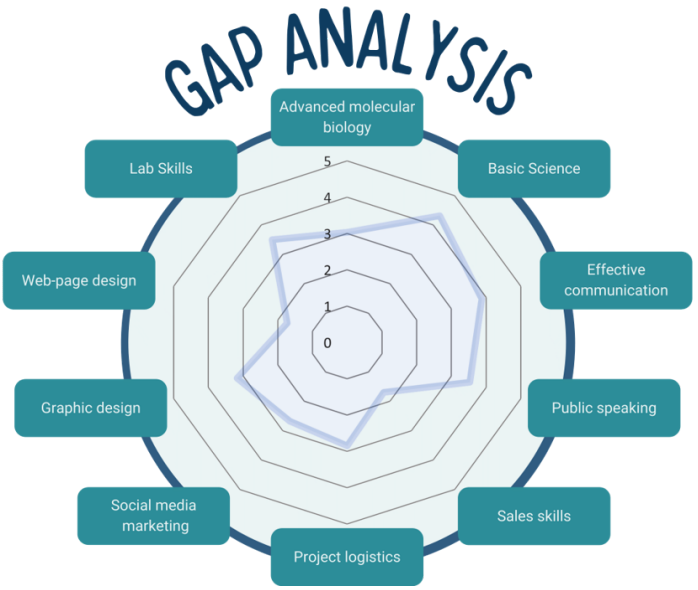


Risk assessment:

We developed a financial risk assessment regarding the Tetl-box, which details the main obstacles for our entrepreneurship project. Also, we proposed plan A, B and C to mitigate these risks.

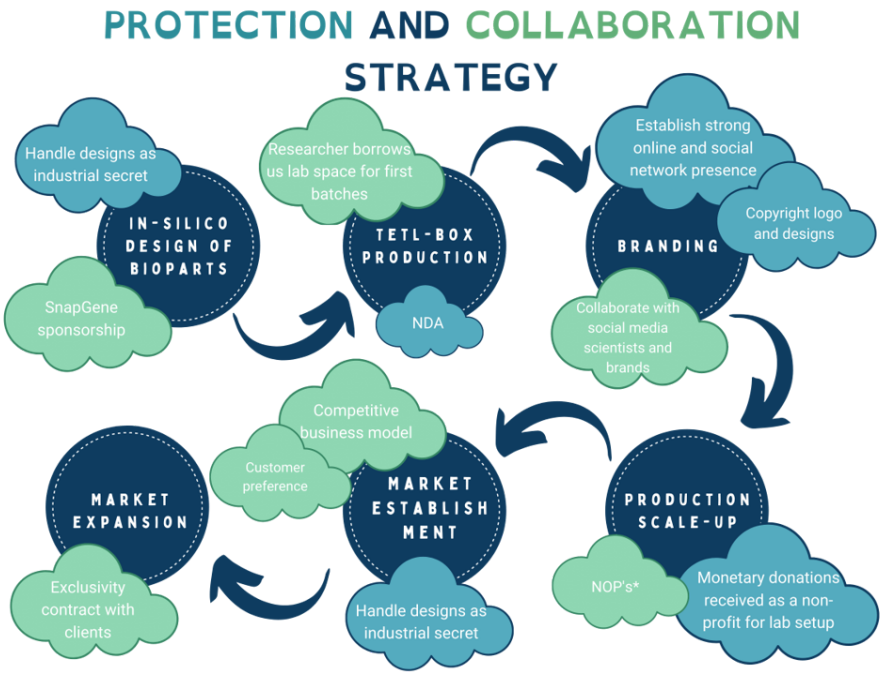
GAP:

To identify the deficiencies of our team, we carried out a **GAP analysis**, which showed us that our team is qualified in the area of basic science, has developed fundamental skills to speak in public, and we also have a great team capable of developing logistics of projects for our business activity. However we have certain skills that the team is still working on, such as web design and sales skills. This is the perfect opportunity to take on a challenge and a new **opportunity to learn**.

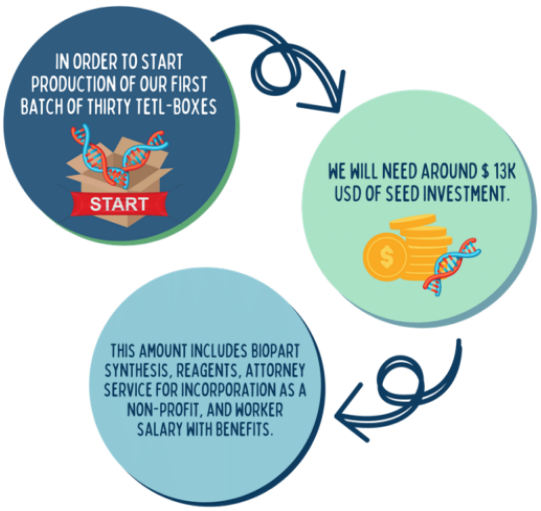


Collaboration and protection strategy:

We elaborated a **protection and collaboration diagram** in order to assess how we are going to collaborate with stakeholders and protect our IP, production scale-up, and market presence.



Seed investment and budget



The production of the Tetl-boxes is as follows: We will synthesize all of the required DNA fragments and bioparts. Then, we will build all of the expression vectors by cutting and pasting DNA and amplifying these parts to have enough integrated genetic circuits and creating a genetic library. These circuits will then be lyophilized and placed in microtubes and these will be placed inside custom-made boxes along with other required documentation like biosafety and usage protocols. This will be the final product that will be shipped to users.

You can read in more detail about this investment by referring to the Seed Investment And Budget document.

Intention letter:

We achieved agreements with educational institutions that are interested in funding our project, sponsoring or doing some kind of donation. We managed to get **three intention letters** from **three different entities**, one being from Ph.D. Ana Laura Torres Huerta and Ph.D. Aurora Antonio Pérez from Tecnológico de Monterrey, a prestigious private Mexican university, the other being the biotechnology department from CINVESTAV, and the last one being María Pía Campot, CEO of Enteria. The CINVESTAV has the intention of providing monetary support for the production of 16 Tetl-boxes under the condition to lower the price to **$230 USD** per box, while Ph.D. Ana Laura Torres has the intention of **providing hardware** like microcentrifuges. María Pía Campot also showed her support for further advancements of our project.

**This documents are not translated to English in order to maintain originality.*

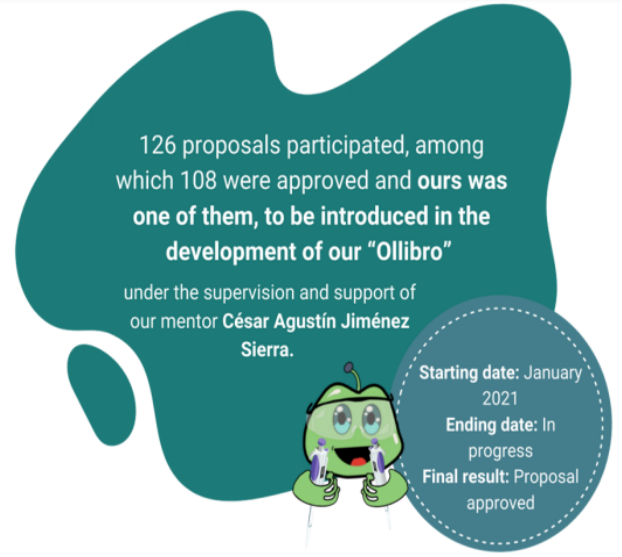
Pitch Deck:

We also elaborated a pitch deck which we plan to present to **accredited potential investors** who also want to make science more accessible in Mexico. This pitch deck has received valuable feedback from **María Pía Campot.**

https://www.youtube.com/watch?v=cE4v_lyTdyM

Ollibro fundraising call

CALL FOR TECHNOLOGICAL DEVELOPMENT OR INNOVATION PROJECTS FOR IPN STUDENTS:
A fundraising call for technological development projects was held at our university to support development and innovation projects.



You can learn more about this achievement by referring to the Ollibro Fundraising Call document (The following document is not translated to English in order to maintain originality).

Donadora:

We coordinated a **crowdfunding** to obtain the necessary resources for the iGEM Design League entrance fee. We **exceeded** our goal with a final **112%** of the total campaign. In addition to meeting this objective, we provided 10% of the total collected to a young Mexican promise **Estrella Salazar**, only 16 years old, current Biotechnological

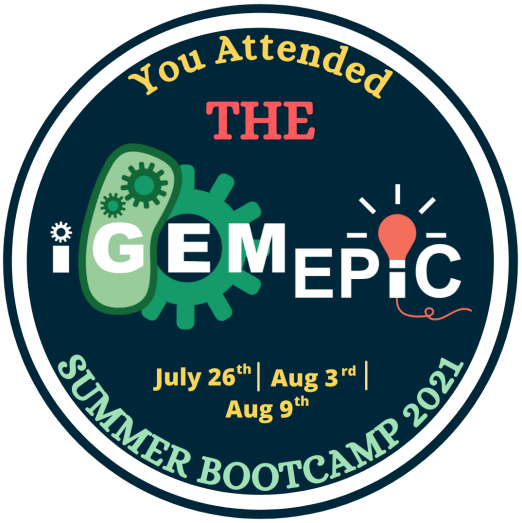
Engineering student, She is currently looking forward to entering the **AEXA International Air and Space Program**, to which she was selected in participating.

EPIC summer bootcamp:

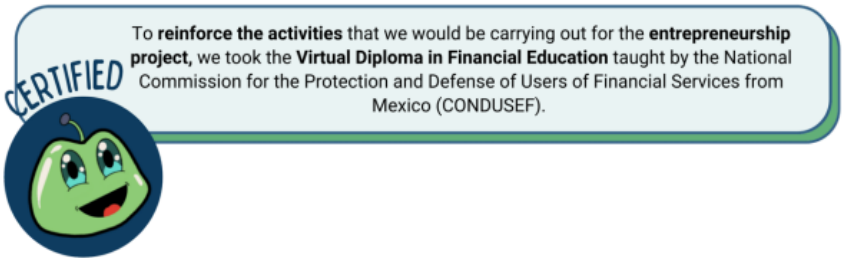
The experience **served us as a helpful resource for the development of our entrepreneurship project,** the iGEM EPIC provided us with support with advice for the essential elements in said project, such as:

- Model Canvas
- Practice and preparation for interviews with our potential clients and sponsors
- Pitch deck

We took all of this as a guide to have the basis of our project ready.



Financial Education online



This is with the objective of learning about finance, since it is key to the entrepreneurship project because it provides timely, useful and relevant information to take actions, project strategies and make the best decisions in favor of the project.

You can read the diplomas obtained in Spanish by consulting the following documents: Financial Education Course (The latter documents are not translated to English in order to maintain originality).

Appendices

OLLIN F&F Risk Assessment

RISK ASSESSMENT							
Which phase in the Gantt chart does the risk apply to?	What is the risk?	What does the risk entail?	How likely is the risk to occur? Use the likelihood axis from the matrix to the right	Interpret the risk rating using the matrix on the right. Mark out the color	What can you do to minimise the risk? Which measures can you take?	What is the back-up plan if your actions to minimize the risk would not be enough? Having a fallback plan B and C gets more important as the risk rating increases!	
Internal Risks (Risks that you can control)							
PHASE	RISK	DESCRIPTION	LIKELIHOOD	RISK RATING KEY	ACTIONS TO MINIMISE RISK	FALLBACK PLAN B	FALLBACK PLAN C
MARKET VALIDATION	No demand for product amongst our target consumers and users	Consumer and user interviews reveal lack of demand & interest for our current product	Improbable	3	Carry out market research at earliest opportunity	Use customer feedback to redesign product, implement new bioparts to the Tetl-box	Pursue alternative venture
PROJECT FINANCING	Don't get sponsors	The project is not attractive to sponsors, so they will not donate to the association.	Improbable	3	Demonstrate that the project works and collect data where the value and need of the project is reflected	Go to foundations or donors abroad	Find another financing model
	Not being able to register as A.C.	For bureaucratic reasons the registration of the team as a Civil Association is not possible	Possible	2	Go to notaries and lawyers to advise the process	Wait the necessary time and carry out the necessary extra procedures	Register as another type of legal person
	Low online store sales	Online stores do not get the expected product sale	Possible	2	Offer clothing and other products that are useful and eye-catching for the customer	Offer other types of products and have a store with more variety and accessible prices	Find another platform to set up a store
BRANDING	Not obtaining favorable results in advertising on social networks	The project turns out not to be attractive to the followers of our social networks	Possible	2	Carry out a series of satisfaction surveys before, after, during and when taking the project to social networks.	Change visual aspects of the project to make it more attractive, talk to marketers	Evaluate the project with the comments received
	Website creation	Due to time, availability of the page or due to any situation, the website cannot be made	Possible	2	Start using the platform, set schedules and have a sketch prepared long before it goes to market	Consult website specialists and designate managers	Search other pages for the realization of the web page
	Do not advertise at seminars, conferences and launch events	Due to time, money or not getting a place in these seminars, conferences etc, we were unable to present our project	Possible	1	Register in advance and schedule	Find contacts to help us enter these conferences and look for more alternatives	Evaluate participating in smaller events and getting invitations to more interesting ones
CONTROL MANUFACTURING	DNA detection at the Mexican border	Due to paperwork or importation laws, our synthesized DNA samples cannot enter the country	Possible	2	Follow all legal necessary paperwork and follow instructions	Look for other countries to synthesize DNA	Look for a way to synthesize DNA in Mexico or through a third party
MARKET EXPANSION	Failure to obtain permission to enter the IPN schools	The project is not accepted by the authorities of the IPN UA	Possible	2	Organize exhibitions to present all the details of the project and persuade them to purchase the product	Carry out feedback surveys and improve the Tetl-box based on it, considering the comments of our users.	Potentiate advertising, being constant with more exhibitions, seminars, etc.
MARKET TAKEOVER	Not covering the schools we expected with the first batch of the Tetl-box	The development of the project is not completed in its entirety and therefore cannot be reproduced in time for distribution	Possible	3	Restructure the project according to previous feedback	Find alternatives to speed up the Tetl-box process	Seeking mentors for Tetl-box improvements
	Failing to cover everything previously planned	We did not get enough sponsors to cover the support and manage to donate to our entire market	Possible	3	Be constant with the contacts until we have enough sponsors to cover our entire market	Make collaborations with teachers so that they are part of the donation team	Expand our advertising to schools or independent users
Not applicable for external risks							
External Risks (Risks outside of your control)							
Phase	Risk	Description	Probability	Risk Rating			
MARKET VALIDATION	Cannot obtain data from users and clients	Surveys and interviews are not being answered by users or do so out of schedule	Possible	2			
PROJECT FINANCING	Sponsors don't have the economic resource to donate to the association	Country falls in an economic crisis, thus possible sponsors don't have any money to donate to our project	Improbable	3			
CONTROL MANUFACTURING	DNA arrives in bad conditions	DNA arrives late or damaged	Possible	2			
MARKET EXPANSION	Tetl-box is not being used in schools	Due to lack of school commitment or student/professor interest, the Tetl-box although functional, is not being used by the users	Improbable	2			
MARKET TAKEOVER	Tetl-box cannot expand to other Mexican states	State governments and schools reject our project	Improbable	3			

OLLIN F&F Interview Cesar

INTERVIEWEE: Ph.D. CÉSAR AZA

INTERVIEWER: ANDRÉS

R: Sí, definitivamente si se pudriera, Como alumno no les toca verlos, pero muchas cosas que usan en la materia, normalmente los vectores que usamos algunas veces son comerciales y otros son los que usamos en proyectos. De manera indirecta, estos que ustedes están haciendo, ya lo hacemos con algunas otras, no con la producción. Definitivamente es viable. En microbiología el técnico laboratorio se encarga de mantener las cepas de bacterias. Acá podría ser algo similar, estar manteniendo estos procesos o lograr estos productos para hacerlo. Si las practicas las adaptamos, sería bueno para alumnos.

- **¿Qué otros reactivos o prácticas le gustarían ver en la caja?**
  R: La transcriptasa reversa, esa sería muy buena tenerla. Esa suele ser cara. Esa en particular se cuida mucho porque viene muy poco y es muy cara.
- **¿Cuál es el proceso que usted sigue para la compra de reactivos e insumos de laboratorio?**
  R: En esto ahí es un poco raro, pero nosotros realmente intervenimos muy poco en el proceso. Por ejemplo, tomando el caso de labs de micro y biote mol los responsables de labs ya tienen los manuales de las practicas que hacemos y ellos se encargan de ver qué es lo que falta y se encargan de comprarlo. Si no decimos nada adicional ellos siguen surtiendo lo mismo en la misma cantidad. Las compras se hacen por año. Nosotros tenemos
- **Si nuestro proyecto llegase a concretarse, ¿le interesaría adquirir la Tetl-box?**
  R: Por el momento, este semestre, yo no estoy dando el lab de biote molecular. Sin embargo, para mi es muy fácil que el próximo semestre diga que yo quiero dar la materia. Si ustedes quieren avanzar el proyecto yo me comprometo a pedir la materia el próximo semestre y poder implementarlo.
- **¿Sufre de limitantes económicas al impartir su clase o realizar sus proyectos de investigación? ¿Qué tanto se ve limitado en la compra de reactivos suficientes?**
  R: Serían más cuestión de equipos. Algo que lo dificulta y se vuelve limitante son los espacios. Sin meternos en detalles, lo de los cupos. Asumiendo que ahorita regresáramos, se tendría que atender 35 alumnos. El espacio físico es una limitante. Si asociamos espacio con lo económico, sería ese lado y también materiales como micropipetas.
- **¿Qué instancias tiene qué consultar para la compra de nuevos reactivos?**
  R: En teoría, nada más con los técnicos de laboratorio, Lo que queda fuera de nuestras manos, sería el presupuesto federal a la escuela. La subdirección académica asigna dinero a las diferentes materias por partidas. Esas partidas lo que se hace es que, si se tiene cierta cantidad dinero, se etiqueta. Se van

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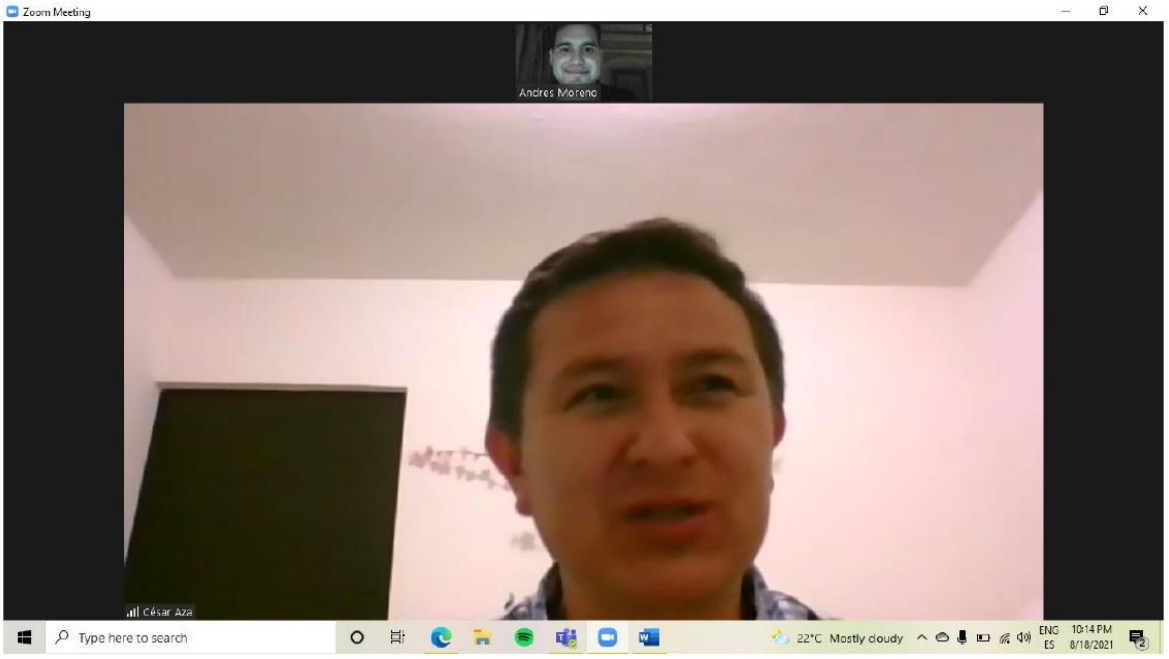
poniendo etiquetas en esas partidas entonces sólo tienes esa cantidad de dinero para la compra de recursos, por lo que sólo tienes una cantidad finita de dinero para comprar reactivos. La limitante es el tiempo con el que solicitamos las cosas para el lab. Yo creo que todo lo que solicitemos nos lo podrán comprar, pero no es tan fácil. Se puede utilizar recurso sobrante para la adquisición de otros recursos (ej: si sobra para la luz, entonces se puede usar para comprar reactivos).

- **Evidencia**



Lean Canvas

Designed for:	Designed by:	Date:	Version:
Ollin SynBio IPN	Finance & Funding	9/12/2021	2

1. Problem

The high cost of reagents for molecular biology experimentation and its applications like synthetic biology, represents an obstacle to teachers, researchers, students, and biohackers to start or continue a career in this field. These economic limitations make education and research in the area of molecular and synthetic biology not a very pursued and popular field in Mexico, placing the country in a disadvantage.

4. Solution

TetI-box a toolbox-inspired low cost kit containing DNA that encodes for the necessary proteins used in molecular and synthetic biology research. Production takes place via a microbial genetic transformation.

3. Unique Value Proposition

With the generation of biofactories using the *TetI-box* the client and/or user will save long-term and short-term reagent costs. With the final obtained products, users will be able to conduct molecular and synthetic biology research in a high school to an overgrad level.

9. Unfair Advantage

Schools in Mexico generally face economic problems when buying science reagents used in molecular biology, as well as problems with time and bureaucracy when trying to import these reagents. With the *TetI-box*, being a product made in Mexico, these problems can be overcome and gain a customer preference over reagent companies like Merck or ThermoFisher.

2. Customer Segments

- School principals
- Biohackers
- Researchers

10. Existing Alternatives

Commercial molecular biology kits from different companies.

8. Key Metrics

- Number of clients
- Number of users
- Quantity of product sold
- User satisfaction level

11. High-Level Concept

We are an alternative from high school to overgrad early stage research.
With the *TetI-box* you have the produce the necessary tools to build different biological systems

5. Channels

- Online store product sales
- Showcase in high school and universities
- Showcase in academic conferences
- Delivery service

2. Early Adopters

- High school and university students and faculty from the life science and engineering area in the Instituto Politécnico Nacional
- iGEM Mexican teams

7. Cost Structure

- DNA synthesis
- *TetI-box* production
- Online store set-up
- Worker wages
- Marketing costs

We will need around $9000 USD as a seed investment and production of the first 30-box batch and incorporation as a non-profit organization, as well as roughly $3000 USD for the production of future 30-box batches.

6. Revenue Structure

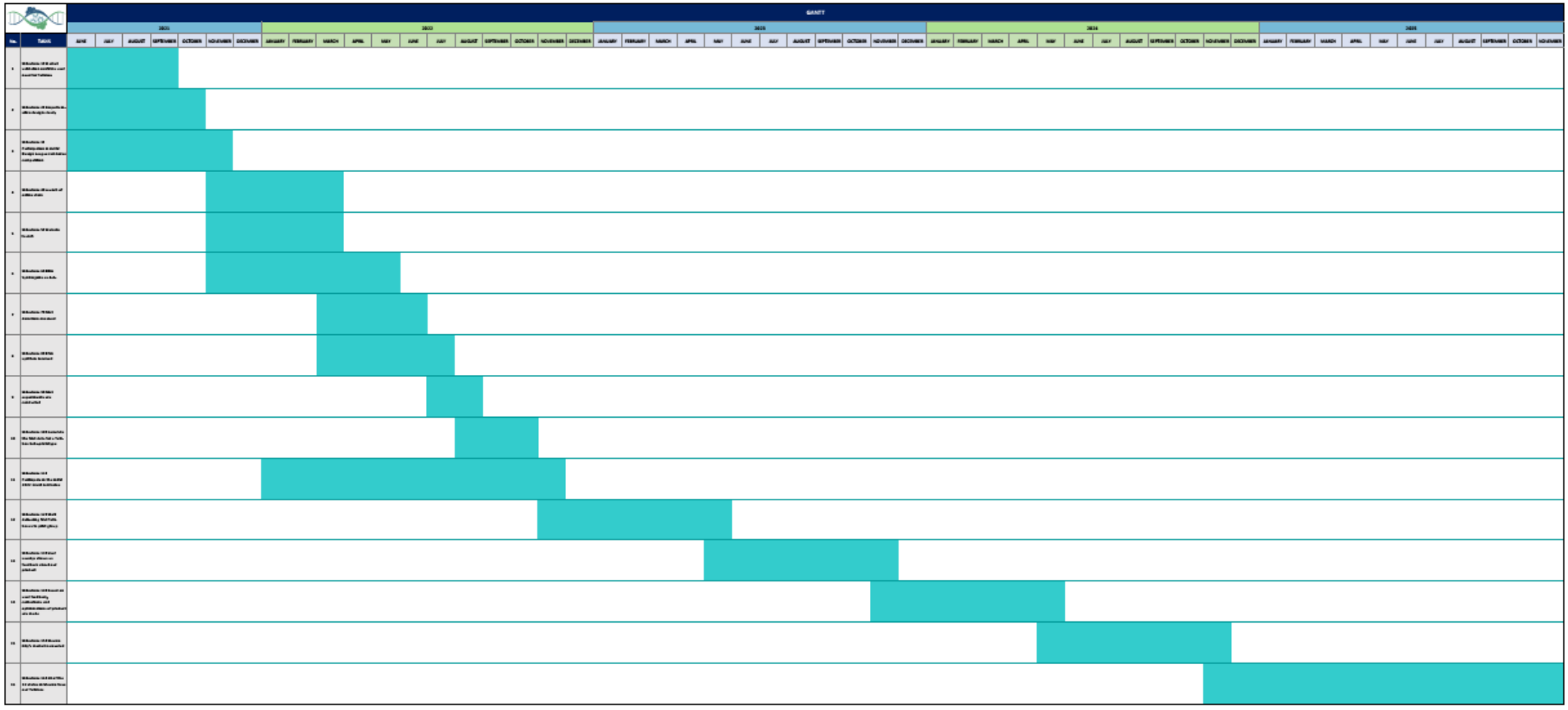
- *TetI-box* sales
- Donations received from companies and philanthropies to Ollin SynBio as a non-profit organization.

Gannt & OKR:

Objectives and Key Results (OKR)

OKR

PHASE	TASK	OBJECTIVE	KEY RESULT(S)	COST (USD)	MATERIALS REQUIRED	CORE SKILLS/CAPABILITIES NEEDED	SKILL/CAPABILITY WITHIN TEAM	OUTSOURCE	COLLABORATE	HIRE
MARKET VALIDATION	Interviews to lecturers and researchers	Receive feedback about the project and their situation in their institution	KR1: 15 interviews made	N/A	Computer for data registry	Communication	☑			
			KR2: At least 90% of interviewees are interested in the TetHbox		N/A					
	Assess our competition	Compare our product with existing alternatives in the market and find our differentiating factors	KR1: The TetHbox has important differentiating factors like cost or potential shelf-life							
			KR2: Competitor ROI found		Computer for data registry and data base elaboration	Research and opportunity finding skills	☑			
	Google forms directed to students	Obtain data from users	KR1: At least 100 students are interested in using the TetHbox							
			KR2: At least 50% of students have a lab in their school with minimal conditions for using the TetHbox		Google Forms	Communication	☑			
			KR3: At least 50% of students have mild knowledge about biology							
PROJECT FINANCING	Fundraising	Fundraise with sponsors and donations	KR1: Obtain the minimum monetary resource to start lab tests	$17,195.40	Contacts and email addresses from possible sponsors/donations	Communication and negotiation	☑	☑	☑	
			KR2: Obtain the minimum monetary resource to make a 30-box batch	$331.00						
	Incorporation as a non-profit	Become an authorized non-profit	KR1: Become legally recognized as a non-profit	$1,600.00	Attorneys	Basic Mexican law knowledge	☑	☑	☑	
			KR2: Sign up for the mexican Servicio de Administración Tributaria	N/A						
	Establish online store	Obtain monetary resources	KR1: Release the online store to the public		T-shirt designs and online store website	Basic business administration knowledge	☑		☑	☑
			KR2: Obtain inventory/capital for sales	$650.00						
			KR3: Sell 50 shirts the first 3 months	N/A						
	Enter fundraising calls	Obtain the resource offered in the call	KR1: Become chosen as beneficiary for the monetary resource offered in the call		R&D Project	Writing	☑			
PRODUCT DEVELOPMENT	In-silico genetic circuit designs	Obtain genetic circuits and mathematical models that describe the project's technical function	KR1: The models describe the correct functioning of the product	N/A	Genetic sequences, programming and bioinformatics software (Matlab, SnapGene, Benchling)	Molecular biology and programming knowledge.	☑		☑	☑
			KR2: The modelled bioparts can be synthesized							
	TetHbox packaging	Establish physical packaging characteristics of the product	KR1: Logo design		Graphic designs, design softwares (Canva), sketches	Creativity and design	☑			
			KR2: Establishment of color pallete							
			KR3: Design of the box as a final product							
	Biosafety analysis of the TetHbox	Inform the user about possible safety risks when using the product	KR1: Information is easy to understand		Consulting services, lab safety documents	Basic lab knowledge	☑		☑	☑
			KR2: No health incidents reports while using the TetHbox are received							
	Protocol description for product use	Offer a guide on how to use the product	KR1: No complaints about lack of clarity about product use are received							
			KR2: Surveys to users suggest high product satisfaction							
BRANDING	Social media marketing	Present our product to our followers	KR1: Obtain at least 100 new followers in the first 3 months of the publicity campaign	N/A	Social media marketing price	Social media marketing, creativity, knowledge in sales	☑		☑	
			KR2: Obtain at least 50 new Instagram post interactions							
			KR3: Obtain at least 5 collaborations with influencers in the first 3 months of the publicity campaign							
	Web page creation	Establish online presence	KR1: Buy internet domain	$12.99/year	Web page programming	Programming, creativity, design, communication	☑		☑	☑
			KR2: Complete the web page design	N/A						
			KR3: Get 10 new followers in social media, thanks to the web page							
	Showcase in seminars and academic conferences	Establish presence in the scientific community	KR1: Get at least 1 sponsor to each event we participate in		Tickets to academic conferences, stand to showcase our product	Marketing, sales knowledge, communication	☑		☑	
			KR2: Assist to at least 6 academic conferences a year	$150/conference/person						
CONTROL MANUFACTURING	Synthesize auto parts and backbones in a foreign company	Obtain DNA fragments that are distributed to schools	KR1: Get a DNA synthesis provider	$0.19/bp	Outsource with DNA synthesizing company (Genscript, IDT, Twist)	Contact with companies	☑	☑		
			KR2: Get the necessary documentation for biopart importation	N/A						
	Amplify the bioparts and backbone	Generate enough boxes to cover demand	KR1: Make biopart amplification	N/A	Technical knowledge in product design and development	Fundamental knowledge in molecular biology lab	☑		☑	
			KR2: Make a deal with a researcher for lab space	N/A						
			KR3: Set up own lab	$60,000.00						
	Packaging and packaging suitable for use	Produce the TetHbox packaging for future distribution	KR1: Contract a box manufacturer	$15.50	Outsource box production	N/A		☑		
MARKET EXPANSION	Provide to schools from the IPN	Start pilot test with 4 IPN schools	KR1: More than half of the students managed to make molecular biology lab experimentation	N/A	Delivery service	Communication, sales	☑		☑	☑
			KR2: At least half the users express product satisfaction through surveys							
			KR3: Close deal with at least half the schools for next year TetHbox provision							
MARKET TAKEOVER	Supply to public high schools and universities that teach biological sciences	Cover the CDMX market	KR1: From donations, provide at least 5 boxes to different schools in a year	$1,310.00	Parcel service nationwide	Sales	☑			
			KR2: Obtain sponsorships from large foundations, such as Slim Foundation	N/A			☑		☑	
	Product launch nationwide	Meet the team's goal of making science more accessible	KR1: Distribute the TetHbox to at least 20 states of the 32 that Mexico has (1 boxes in each state considered)	$14,480.00		Marketing, ventas, divulgación científica	☑			
			KR2: Have a presence in at least one high school and one university in the states where the TetHbox will be distributed	N/A						☑
			KR3: Surveys show that more users were able to learn about synthetic biology and molecular biology thanks to the use of the TetHbox				☑		☑	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Seed Investment and Budget

OLLIN SYNBIO

Seed Investment

Concept:	Genes						
MATERIALS		**Unit**	**$/bp**		bp	$	
Primers		BP	$0.83	*	20.00	$630.80	
Ligase		BP	$0.19	*	1,568.00	$297.92	
Pfu pol		BP	$0.19	*	2,432.00	$462.08	
Taq pol		BP	$0.19	*	2,730.00	$518.70	
Bst pol		BP	$0.19	*	2,735.00	$519.65	
Peroxidase		BP	$0.19	*	1,160.00	$220.40	
GFP		BP	$0.19	*	720.00	$136.80	
RFP		BP	$0.19	*	706.00	$134.14	
XbaI		BP	$0.19	*	725.00	$137.75	
NotI		BP	$0.19	*	1,256.00	$238.64	
EcoRI		BP	$0.19	*	944.00	$179.36	
SpeI		BP	$0.19	*	1,034.00	$196.46	
PstI		BP	$0.19	*	1,076.00	$204.44	
AmpR/caspase vector		BP	$0.19	*	8,925.00	$1,695.75	
KanR/caspase vector		BP	$0.19	*	8,686.00	$1,650.34	
Banana		BP	$0.19	*	1,581.00	$300.39	
aeBLUE		BP	$0.19	*	717.00	$136.23	
DNAsa		BP	$0.19	*	1,256.00	$238.64	
RNAsa		BP	$0.19	*	3,227.00	$613.13	
Protease		BP	$0.19	*	1,058.00	$201.02	
SUBTOTAL:	**Genes**						**$8712.64**

Concept:	Reagents					
MATERIALS		**Unit**				
Agarose		Piece	$704.00	*	1.00	$704.00
EcoRI		Piece	$62.00	*	1.00	$62.00
PstI		Piece	$67.00	*	1.00	$67.00
T4 ligasa		Piece	$64.00	*	1.00	$64.00
kit PCR		Piece	$540.00	*	2.00	$1,080.00
TBE		Piece	$45.76	*	1.00	$45.76
SUBTOTAL:	**Reagents**					**$2022.76**

Concept:	Incorporation costs					
Services		Unit				
1N	Lawyer	Full service	$1,500.00	*	1	$1,500.00
SUBTOTAL:	**0**					**$1500.00**

TOTAL SEED INVESTMENT: **$12,235.40** **USD**

Direct Costs

Concept:	Reagents					
MATERIALES						
Agarose		Piece	$704.00	*	1.00	$704.00
kit PCR		Piece	$540.00	*	2.00	$1,080.00
TBE		Piece	$45.76	*	1.00	$45.76
SUBTOTAL:	**Reagents**					**$1829.76**

Concept:	Genes					
MATERIALES						
Primers		PB	$0.83	*	20.00	$630.80
SUBTOTAL:	**Genes**					**$630.80**

Concept:	Wage					
Labour costs						
1T	1 Technician	Working day	$5.65	*	4.0	$22.60
SUBTOTAL:	**Labour costs**					**$22.60**

TOTAL DIRECT COSTS: **$2,483.16** **USD**

Business proposal		
Total (4 years):	USD	$6,005.92
Initial capital:	USD	$1,039.60
Total of cycles:		0.21
Time in years for ROI		0.42
Unit cost	USD	$82.77
Profit	USD	$165.54
Selling price	USD	$248.32
Profit after 2 year cycle	USD	$4,966.32

Financial Education Course

HACIENDA
SECRETARÍA DE HACIENDA Y CRÉDITO PÚBLICO

CONDUSEF

LA COMISIÓN NACIONAL PARA LA PROTECCIÓN
Y DEFENSA DE LOS USUARIOS DE SERVICIOS FINANCIEROS
OTORGA EL PRESENTE

DIPLOMA

A

CARLA JANNET RENTERIA HERNÁNDEZ

POR HABER ACREDITADO EL DIPLOMADO EN
EDUCACIÓN FINANCIERA, CON UNA DURACIÓN
DE 150 HRS, IMPARTIDO POR LA CONDUSEF.

GENERACIÓN 39, MAYO – AGOSTO 2021

OSCAR ROSADO JIMÉNEZ
PRESIDENTE DE CONDUSEF

https://diplomado.condusef.gob.mx/diploma/abreDiploma.php?codigo=110354x3955a0abb4d150b2f586bdd7cb0abadfx94fcb14d8

CIUDAD DE MEXICO, AGOSTO 2021

HACIENDA
SECRETARÍA DE HACIENDA Y CRÉDITO PÚBLICO

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LA COMISIÓN NACIONAL PARA LA PROTECCIÓN
Y DEFENSA DE LOS USUARIOS DE SERVICIOS FINANCIEROS
OTORGA EL PRESENTE

DIPLOMA

A

DANIELA PÉREZ CORTES

POR HABER ACREDITADO EL DIPLOMADO EN
EDUCACIÓN FINANCIERA, CON UNA DURACIÓN
DE 150 HRS, IMPARTIDO POR LA CONDUSEF.

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CIUDAD DE MEXICO, AGOSTO 2021

Intention letters:

*

Ciudad de México, México a 07 de octubre del 2021

Cinvestav – IPN
Departamento de Biotecnología
Av. Instituto Politécnico Nacional 2508
San Pedro, Zacatenco, Gustavo A. Madero 07360,
Ciudad de México, CDMX

Asunto: Carta de intención

Estimada Dra. Beatriz Xoconostle Cázares:

Por medio de la presente me permito informarle que el grupo estudiantil "Ollin SynBio - IPN" representado por el Instituto Politécnico Nacional a través de la Unidad Profesional Interdisciplinaria de Biotecnología, Unidad Profesional Interdisciplinaria de Ingeniería campus Guanajuato y la Escuela Nacional de Ciencias Biológicas, mantiene la firme **intención de confirmar** el apoyo de la donación monetaria equivalente a la elaboración de dieciséis unidades del producto que elaboran, denominado de ahora en adelante ***TetI-box,*** con un precio unitario de $230 USD más inflación. Asimismo, el Colegiado del Departamento de Biotecnología del Cinvestav, de ahora en adelante, **donataria**, mantiene la firme **intención de donar** el equivalente monetario para la elaboración de dieciséis unidades *TetI-box*. Ollin SynBio - IPN se compromete a donar al menos dos de estas dieciséis unidades a dos escuelas nivel medio superior o superior cualquiera ubicadas en municipios de Colima, México.

1. **Proyecto**: *TetI-box* es inspirado en una caja de herramientas la cual contiene en su interior veintiún fragmentos de ácido desoxirribonucleico en formulación liofilizada los cuales codifican para proteínas y enzimas utilizadas en experimentación en biología molecular. El fin de estos fragmentos es llevar a cabo una transformación genética en una bacteria *Escherichia coli* para la posterior producción y purificación de los productos genéticos.

2. **Efectos no vinculantes**: En virtud de que el proyecto se encuentra en una etapa de investigación y validación, tanto Ollin SynBio - IPN como la donataria, reconocen que esta carta no tendrá efectos vinculantes entre estos, ni respecto de terceros, por lo que será facultativo de la donataria optar por proceder o no con relaciones posteriores una vez que el producto se encuentre listo para producción. Ollin SynBio - IPN se compromete a notificar a la donataria una vez que: (i) haya concluido el proceso de investigación y (ii) *TetI-box* se encuentre listo para producción.

En caso de estar de acuerdo, agradecemos la firma en los siguientes espacios, en señal de aceptación del contenido, atentamente

CONFIDENTIAL

CONFIDENTIAL: Representante del Colegiado del Departamento de Biotecnología del Cinvestav

CONFIDENTIAL

Andrés Moreno Morales: Representante del área de finanzas de Ollin SynBio - IPN

Contacto: funding@ollinsynbio.org

* Personal data is omitted at the explicit request of the donor.

Tecnológico de Monterrey

Estado de México, a 10 de octubre de 2021

Ollin SynBio – IPN
A quien corresponda

Asunto: carta de intención de colaboración

Por medio de la presente hago constar mi interés en establecer una colaboración con su grupo estudiantil para desarrollar y proporcionar ejemplares de algunos equipos de laboratorio. En conjunto con otros profesores de mi departamento, hemos creado versiones miniaturizadas de algunos equipos de uso frecuente en biología molecular, a este proyecto le llamamos "HomeLab". Esta iniciativa se desarrollo para brindar la oportunidad de desarrollar experimentos en casa de nuestros estudiantes durante la pandemia. Tenemos interés de que nuestra iniciativa tenga mayor impacto y que escuelas de bajos recursos puedan tener acceso a estos equipos de tal forma que se incentive el interés en la ciencia de más estudiantes mexicanos. Los equipos con los cuales deseamos establecer colaboración son: mini centrifuga, mini cámara de electroforesis y mini transiluminador.

Considero que nuestros equipos serán una gran aportación al kit que ustedes se encuentran desarrollando "Tetl box". Sumando esfuerzos lograremos mejorar la educación en nuestro país y cada vez más estudiantes tendrán un acercamiento a la biología sintética.

Sin más por el momento y en espera de definir los detalles de esta colaboración, me despido con un cordial saludo.

Atentamente

Dra. Ana Laura Torres Huerta
Profesor
Depto. Bioingeniería
Escuela de Ingeniería y Ciencias
ITESM-CEM
atorresh@tec.mx

Montevideo, 21 de octubre de 2021

Asunto: Carta de interés

Estimado equipo Ollin SynBio

P R E S E N T E

Tras haberme reunido con ustedes y entender el proyecto "Tetl-Box" para que estudiantes de bachillerato y universidad logren una mejor calidad en su educación. Por medio de la presente carta, deseo informar y manifestar mi INTERÉS en apoyar su proyecto central, así como los proyectos satélites que lo conforman.

Confío en que el proyecto "Tetl-Box" así como sus proyectos satélites, permitirá reducir de manera importante las inequidades y contribuir al desarrollo científico y tecnológico de estudiantes de todo Latino América impulsando de manera importante su interés y curiosidad, esperando que en el corto y mediano plazo esto contribuya a una tener una sociedad más preparada para enfrentar los retos planteados por los Objetivos de Desarrollo Sustentable de la Agenda 2030 de las Naciones Unidas.

Aprovecho la ocasión para enviarles un cordial saludo.

A T E N T A M E N T E

María Pia Campot

Contacto: funding@ollinsynbio.org