

POETICIDE: Thunbergia alata

control through SynBio

1. Project Description Section

1.1. Project Summary

SynthEIA is a research group made up of biotechnology, biomedical and administrative engineering, and physics students from EIA University here in Medellín, Colombia, this is the first time we're joining iGEM Design League, in its 2023 version.

Our team consists of a team advisor, the director of biotechnology engineering of our university, two mentors, a biological engineer, Master of Biotechnology and PhD candidate in biotechnology, and a bacteriologist and clinical laboratory technician with master's and PhD in biology; and 15 team members, divided into three committees. The science communication and graphic design committee, also responsible for videos, social networks, and art; the human practices and collaborations committee, which is integrated with biosafety and biosecurity, regulations and policies, and entrepreneurship; and, finally, the research committee, in charge of the genetic circuit design, its standardization and optimization and the build and test protocols.

Remaining our problem, biological invasions are one of the main reasons of biodiversity loss worldwide. *Thunbergia alata*, commonly known as black-eyed Susan vine, a climbing plant native to the east coast of Africa, that was introduced in America in the 19th century for ornamental purposes, has developed a great propagation capacity in the areas where it has been introduced and is currently considered an aggressive invasive plant, mainly in tropical and subtropical areas around the world.

Among the characteristics that position it as a super plant are: accelerated development and dispersal mechanisms that favor its proliferation; presence of parenchyma that surrounds the conductive tissues and allows the conduction of water and sugar transfer, triggering a better adaptation to climatic conditions and greater resistance to mechanical damage; the presence of lignified cells that provide an aggressive vegetative growth; its habit of climbing through various supports and colonizing them quickly until covering them entirely from dense layers of stems and leaves; the presence of arbuscular mycorrhizal

fungi that gives it greater adaptability and ease for its growth, for the favorability that they provide to the absorption of nutrients from the soil.

In the local context, *T. alata* is considered one of the 10 most problematic invasive plant species in Colombia and has managed to colonize the vegetation of the high plateau from Eastern Antioquia, generating various negative effects on Andean ecosystems and production systems.

A gene silencing strategy based on small interfering RNA (siRNA) is proposed for the control of *Thunbergia alata*, where the *FATA*, *FATB1*, *FATB2*, and *KASII* genes will be targeted. Highly potent recombinant 21-nucleotide (nt) siRNAs produced in *Escherichia coli*, specific for certain regions of the transcripts of these genes, conjugated to positively charged carbon dots nanostructures that facilitate high permeation rates into *T. alata* leaf cells and prevent RNase-mediated degradation, will be used. In addition, a spray method is proposed as a mechanism for siRNA introduction.

References and other indexes can be reached in [this link](#).

1.2. Track

Environment and Biodiversity



1.3. Promotional Video

You can watch our promotional video on our [YouTube channel](#) or in [this folder](#).

1.4. Project Presentation Video

You can watch our promotional video on our [YouTube channel](#) or in [this folder](#).

1.5. Team and Attributions

Human Practices and Collaboration commettee



Juliana Calderon Dominguez *Isabella Montoya Valencia* *Andrea Arredondo Restrepo*



Mariana Beltrán Londoño *Isabel Morales Posada* *Estefanía Cardona Ángel*

Science Communication and Graphic Design commettee



Santiago Agudelo Velásquez *Manuela Vanegas Marín* *Carolina Gámez Herazo*



Maria Fernanda Zapata Jaramillo *Camila Sabas López* *Maria Camila Palacio Hernández*

Leaders



Main advisor
Juan Pablo Arias Echeverri

Team leader
Simón Gil Castaño



Mentor
Simón Villegas Velásquez

Mentor
Isaura Patricia Torres Gómez

Research commettee



Miguel Osorio Solórzano *Melissa Moreno Ruiz*

Figure 1. Team members, mentors and advisors divided in their respective committees

2. Design and Results Section

2.1. Design Roadmap



Figure 2. SynthEIA's design roadmap

The implementation of a design methodology proved to be of great assistance in the development of the project at hand. It not only allowed for agile work but also encouraged the entire team to take an active role in decision-making regarding the problem and the design of the solution prototype.

Design Sprint is a design methodology that combines the best elements of Design Thinking, agile development, product strategy, and more, aiming to quickly gather feedback from experts on the idea to be implemented (Mauricio Angulo S, 2019).

Our team was interested in this methodology because its focus is on ideation and learning, which became crucial for this delivery. We didn't want to immediately select the first proposed solution; instead, we wanted to involve every team member so that, through a voting process, we could choose the solution that aligned best with our ideals and ambitions, drawing on positive aspects from various ideas.

The entire project's development using this methodology was made possible with the support of experts and facilitators. They not only provided feedback based on their knowledge to modify and enhance the design but also ensured that we stayed on course with our primary objective: finding an effective solution to address a problem affecting our entire region.

Furthermore, we aimed to modify the way we implemented this methodology by not condensing it into a 5-day sprint but dividing it into 7 sessions. During each session, the entire team shared their ideas at every stage of the Design Sprint. Each session was focused on ideation for the upcoming stages of the competition, facilitating a collaborative and active environment where everyone was engaged. Additionally, between sessions, each team member carried out their individual tasks, assignments, and responsibilities related to iGem Academy.

This design methodology allowed us to arrive at collective ideas, as in each session, we shared our progress and discussed possibilities for advancing our project in preparation for the different competition deliverables. The structured timing of each session aligned strategically with the deadlines for each deliverable.

2.2. Genetic Circuit Design

I. Proof of concept

A gene silencing strategy based on small interfering RNA (siRNA) is proposed for the control of *Thunbergia alata*, where the *FATA*, *FATB1*, *FATB2*, and *KASII* genes will be targeted. Highly potent recombinant 21-nucleotide (nt) siRNAs produced in *Escherichia coli*, specific for certain regions of the transcripts or messenger RNAs (mRNAs) of these genes, conjugated to positively charged carbon dots nanostructures that facilitate high permeation rates into *T. alata* leaf cells and prevent RNase-mediated degradation, will be used. In addition, a spray method is proposed as a mechanism for siRNA introduction.

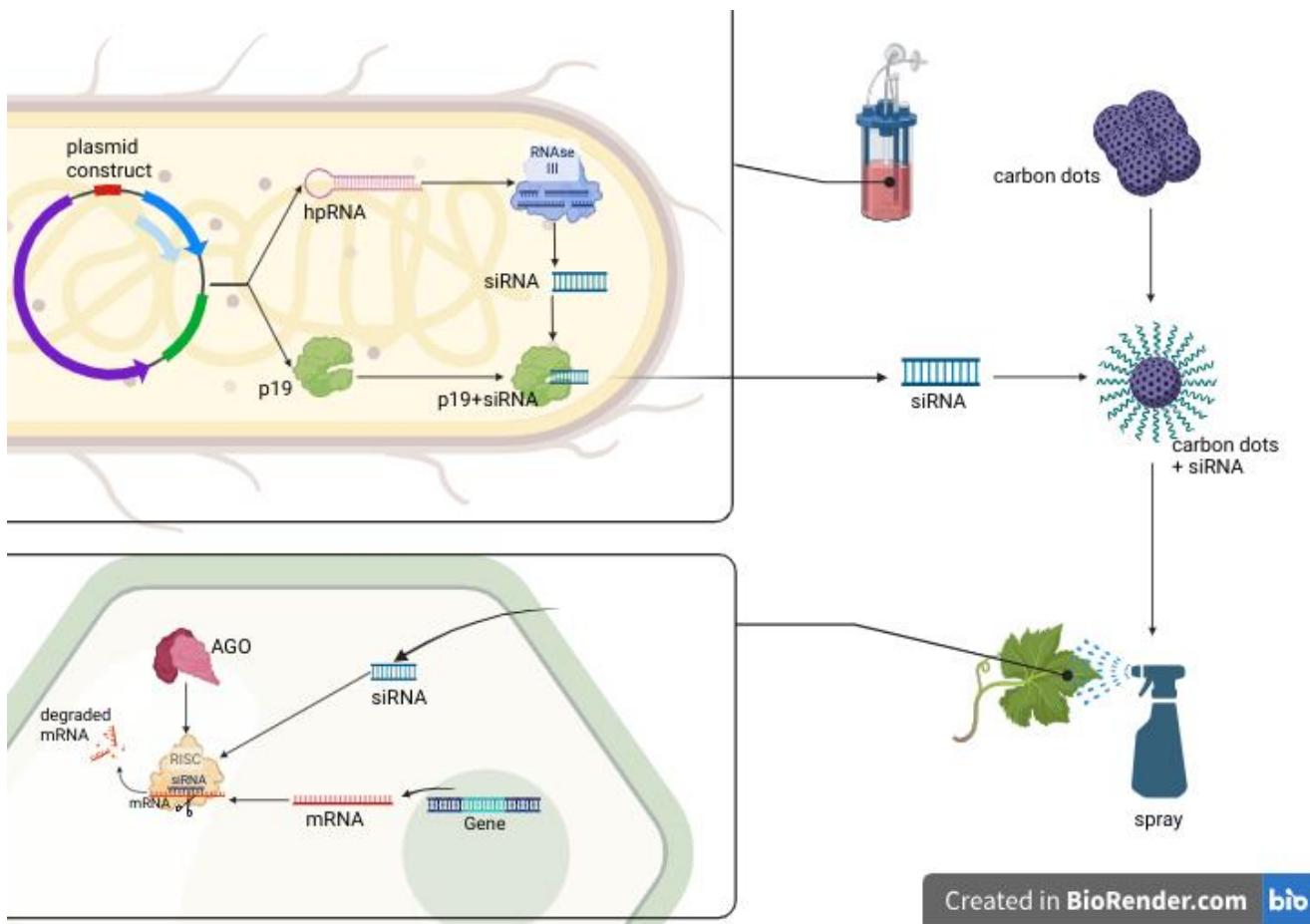


Figure 3. Graphic summary of *T. alata* control strategy

In the proposed vector, a construct for the transcription of hairpin RNAs (hpRNAs) of approximately 250 nt, specific for certain regions of each target gene, will be inserted, from which highly potent siRNAs will be produced in *E. coli* cells through the action of RNases III, characteristic DICER-type enzymes of this prokaryotic organism (Yang et al., 2004). On the other hand, it is also proposed to encode the p19 protein, useful for the stabilization of the siRNAs produced (Cheng et al., 2007), to be subsequently purified for their direct application on *T. alata* leaves through a mechanism of spraying. Figure 1 illustrates the proposed strategy, with some concepts that will be expanded in the following sections.

II. State of the Art

Different approaches have been evidenced in the literature for the utilization of RNA interference (RNAi) for the silencing of some genes of interest, demonstrating its potential for a possible control of weeds or unwanted plants. Dalakouras et al. (2016) propose a method of high-pressure spraying of siRNAs for gene silencing, demonstrating efficient silencing of the transgene analyzed at both systemic and local levels, proving to be a method as efficient as the introduction of siRNAs by bioballistics, but at the same

time, much simpler and more convenient. Likewise, similar approaches exist, but with the use of nanostructures that facilitate the delivery of RNAs and avoid their degradation; Mitter et al. (2017) propose the use of clay nanostructures in leaf-like conformations for a sustained release of double-stranded RNAs (dsRNAs) that confer plant protection against certain viruses, where a high protection rate was shown, outlining this technique for the silencing of specific genes. Zhang et al. (2019) propose the use of DNA nanostructures conjugated to 21 nt siRNAs for green fluorescent protein (GFP) silencing in plants.

Schwartz et al. (2020) proposed the use of carbon dots as an efficient alternative for gene silencing. In this study, siRNAs of 22 nt in length, specific for a region of the GFP transgene inserted in *Nicotiana benthamiana* and *Solanum lycopersicum* are used to appreciate the silencing rates easily by analyzing the fluorescence of the leaves. In addition, the silencing of some endogenous genes in *N. benthamiana* is analyzed by quantifying the transcripts. As a result, a high rate of gene silencing was demonstrated for GFP and even for the endogenes *CHLH* and *CHLI*, showing a decrease in transcripts of up to 79 % by reverse transcription quantitative PCR (RT-qPCR), after 5 days of treatment.

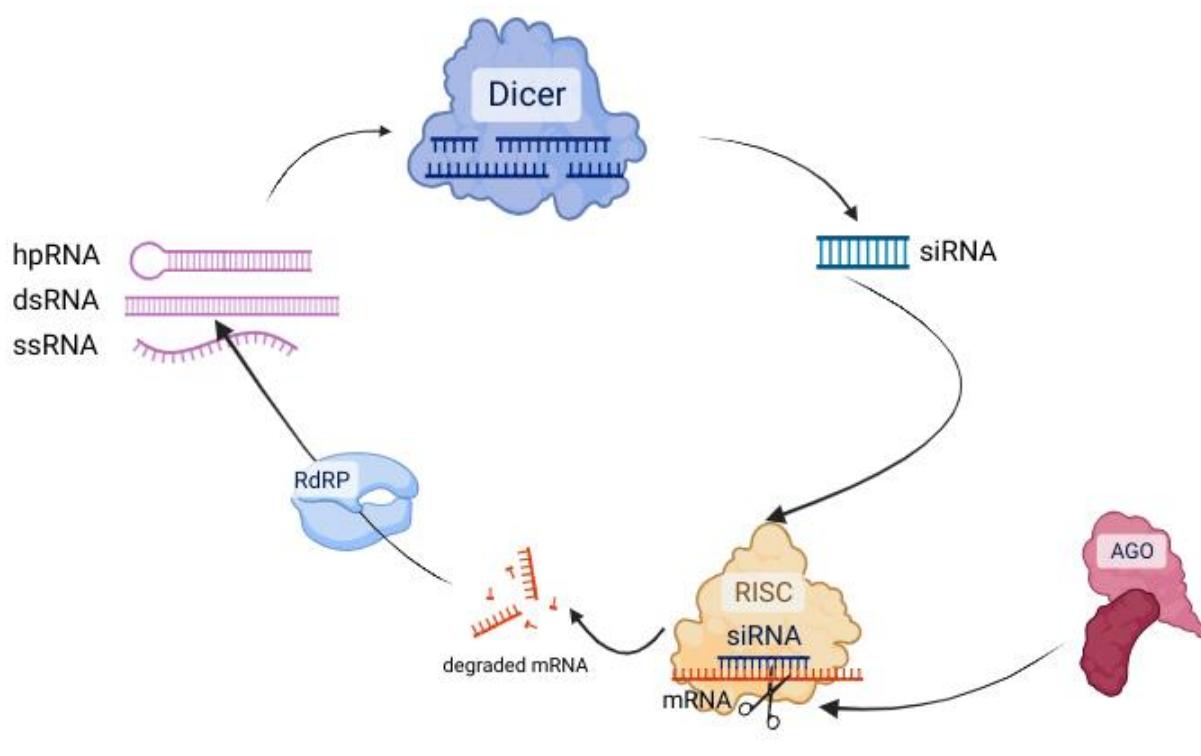
It has been shown that carbon dots emerge as a more environmentally friendly alternative to other nanoparticles such as quantum dots, since they are structured mainly with carbon, allowing greater biocompatibility, resulting in being less harmful and toxic to the environment (Liu et al., 2012), 2012), and with high potential due to their small size (less than 10 nm usually). Carbon dots are biocompatible and versatile nanoparticles that can be composed with different structures and properties depending on the elaboration procedure used; there are different methods, such as Top-down, or Bottom-up, where the former uses carbon compounds such as graphite as a substrate, while the bottom-up method uses simpler carbon precursors such as monomers and polymers, allowing to obtain differentiated structures according to the desired application, such as for cancer treatment, drug delivery, crop treatment, etc. (Yao et al. , 2019).

In addition, the use of carbon dots as an efficient vehicle for the transport of genetic material is reported, by manufacturing them from polyethylenimine (PEI), a commercially inexpensive polymer which, thanks to its high content of amino groups, gives way to the formation of positively charged nanoparticles, facilitating the adhesion of genetic material (such as DNA or siRNA) to them (Liu et al., 2012; Li et al., 2021). Likewise, in tests carried out to produce carbon dots from PEI, efficient nanoparticles have been obtained, with high transfection rates to plant cells and low cytotoxicity associated with their formation (Li et al., 2021).

2.2.1. Abstraction

I. RNAi: Natural mechanism

RNAi is a specific mechanism of eukaryotic organisms, useful for the silencing of endogenous or exogenous genes, both at the level of transcription and translation, transposon silencing or even defense against viruses (Hendrix et al., 2021). It has been evidenced that this natural silencing process can be induced from the presence of exogenous RNAs, such as long dsRNAs (long dsRNAs), hairpin RNAs (hpRNAs), single-stranded RNAs (ssRNAs) or siRNAs. In this mechanism, dsRNAs and hpRNAs are recognized and processed for the formation of secondary siRNAs, microRNAs, or some other short RNAs through the action of DICER-type ribonucleases (Dubrovina & Kiselev, 2019).



Created in BioRender.com

Figure 4. Diagram of the natural process of RNAi

The secondary siRNA fragments generated, approximately 21 - 22 nt long, are subsequently incorporated into Argonaute-like proteins (AGO), RNA-binding proteins with specificity for microRNAs and siRNAs, such as AGO1 and AGO2, for which these RNA segments function as a "guide" for the detection of the mRNA to be silenced, thanks to the complementarity of bases. When these RNAs are incorporated into

the AGO proteins, they form, with other proteins, the RNA-induced silencing complex (RISC), which facilitates gene silencing by repressing the translation processes, or by binding to the mRNAs for their cleavage, generating fragments that will be degraded or that can serve as a template for the generation of new dsRNAs by RNA-dependent polymerases (RdRP), which will again allow the production of new secondary siRNAs, thus forming a cyclic process of mRNA silencing, and increasing the efficiency of this mechanism for gene silencing (Hung & Slotkin, 2021). For a better understanding of this process, it is recommended to watch: [RNA interference – Nature Videos](#).

Different studies have applied RNAi-based silencing techniques. Among them is the direct application of siRNAs, which, when designed with complementarity to mRNAs, facilitate their degradation (Senthil-Kumar and Mysore, 2011). Despite their small size and vulnerability to degradation, siRNAs allow versatility when considering methods to deliver them to intact plant cells, such as the use of different vectors or nanostructures that facilitate their entry into the cells and preserve their integrity, thus achieving the formation of efficient RISC complexes that specifically silence the genes of interest (Demirer et al., 2020).

The present project proposes to make use of this mechanism at the level of the siRNAs, after producing the hpRNAs, and these will be processed by the RNAase III enzymes in *E. coli*, from which it is expected to obtain diverse sequences for highly potent siRNAs for the silencing of the four genes in question. The silencing mechanism will consist in the conjugation of these siRNAs introduced to the AGO proteins for the formation of the RISC complex in *T. alata* leaf cells, so that this complex will localize the mRNAs of FATA, FATB1, FATB2 and KASII, degrading them and thus achieving significant silencing.

II. *Thunbergia alata* and FA-synthesis-related genes: metabolic importance and chosen sequences

Literature has limited information on *T. alata*, especially on sequencing data, however, partial mRNA sequences were found for some genes related to fatty acid (FA) metabolism, of great metabolic importance for plant species and with regions sufficiently differentiated from other species to extract the coding sequence options proposed for the design of the genetic circuit.

To ensure selectivity and choose sequences, alignment with BLAST was performed with the NCBI nucleotide collection database for each target gene, with the aim of finding regions of approximately 250 bp with little or no overlap with other organisms; the fragments chosen were again locally aligned to verify their specificity. All BLAST analyses were performed with default parameters and excluding *T. alata*.

FAs are major components of plant cell membranes and organelles, and play an essential role in their structure, development, signaling and stress response (Wu & Hou, 2010). They are precursors of other complex molecules such as waxes; in addition, some are converted into other messenger compounds that have fundamental roles in signal transduction pathways (Bonaventure et al., 2003; Zheng et al., 2005; Roudier et al., 2010). Moreover, they are the substrate for the synthesis of storage lipids such as triacylglycerols (TAGs), specifically in the cotyledon or endosperm of seeds, where they are important for their germination (Durrett et al., 2008; Dyer et al., 2008). Additionally, they have key functions in plastid development, as they are an intermediate step in the supply of polar lipids relevant to chloroplast division and development (Wu & Hou, 2010).

- **KASs**

The first step in the de novo biosynthesis of FA is catalyzed by acetyl-CoA carboxylase, which converts acetyl-CoA to malonyl-CoA (Guchhait et al., 1974; Gornicki and Haselkorn, 1993), which is subsequently condensed by a group of protein β -ketoacyl-[acyl carrier protein (ACP)] synthases or KASs, FA chain elongation initiating enzymes (Yang et al., 2016).

These KASs are crucial for carbon chain condensation and chain elongation from 4 (C4) to 18 (C18) carbon atoms. In plants, several specific types of KASs, including KASI, KASII, KASIII and KASIV have been characterized in various species (Yang et al., 2016), of which the first three have been shown to be essential for plant metabolism. KASI and KASII are the condensing enzymes for carbon chain elongation from C4 to C14 (Wu & Hou, 2010). Specifically, KASII is a key enzyme that catalyzes the last condensation reaction of palmitoyl-ACP to stearoyl-ACP (Murphy, 2020), and in general the conversion of FA from C16 to C18, which are involved in different cellular processes, such as the biosynthesis of triacylglycerols, glycerolipids, and phospholipids (Ye et al., 2009), important for cell signaling, formation of very long chain FA (VLCFA) for cuticular waxes and plant development, and for conversion to hormones responsible for stress response (Ohlrogge and Browse, 1995).

When analyzing the sequenced region of the mRNA belonging to KASII (NCBI accession number OL757548.1) using the BLAST tool, the alignment map in Figure 5A is obtained, where it is observed few matches in the initial region of the transcript of approximately 300 base pairs (bp).

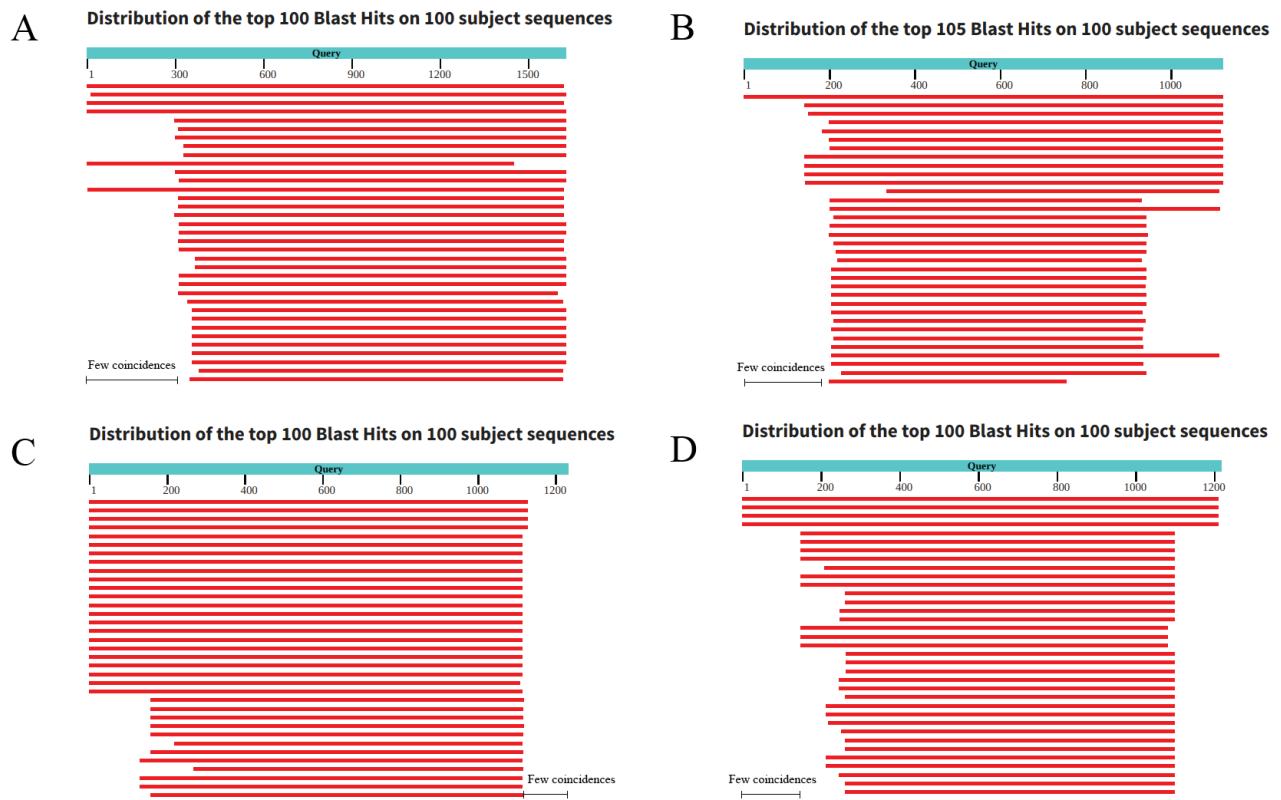


Figure 5. Alignment map by BLAST tool for mRNA: (A) KASII; (B) FATA; (C) FATB1; (D) FATB2.

Taken from BLAST on-line tool (Madden, 2002).

From the alignment obtained, a 250 bp segment was selected at the beginning of the KASII mRNA. For this, a new alignment analysis was performed with BLAST, obtaining as a result what is shown in Figure 6A, demonstrating the presence of two local alignments of short length, with the organisms *Berberis vulgaris* and *Hevea brasiliensis*.

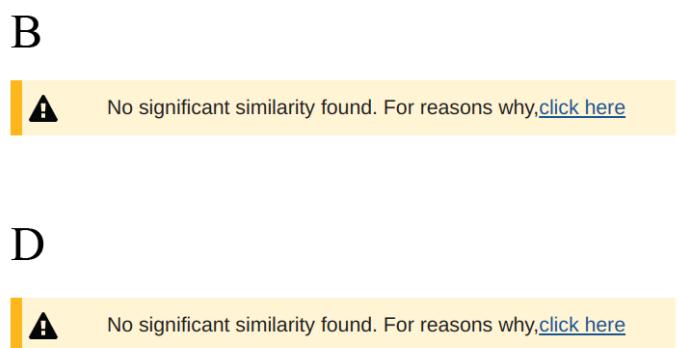
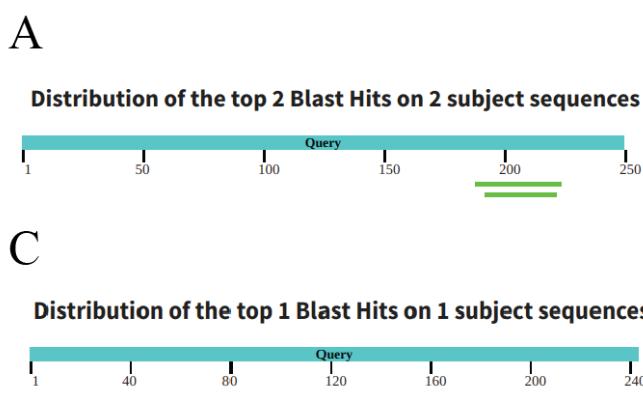


Figure 6. BLAST for selected segments of the sequences of: (A) KASII (graphic summary); (B) FATA; (C) FATB1 (graphic summary); (D) FATB2. Taken from BLAST on-line tool (Madden, 2002).

However, as these alignments are short, they do not represent a significant risk. To achieve significant silencing, it is necessary to form siRNAs with high complementarity to the target mRNAs, and at the time of production of the multi-potent siRNAs, different complementary sequences will be generated in the range of 250 nucleotides selected, with a low probability of formation of significant quantities for segments 100 % complementary and specific to the local alignments with these two organisms.

- **FATs**

Now, the de novo synthesis of GA is terminated by protein acyl-ACP thioesterases, which catalyze the hydrolysis of the thioester bond of the acyl-ACP synthesized by fatty acid synthases (Salas & Ohlrogge, 2002; Aznar-Moreno et al., 2018). Acyl-ACP thioesterases are enzymes, targeting plastids, encoded by nuclear genes (Yuan et al., 1996; Mayer & Shanklin, 2005), and classified into two families based on the results of their sequence alignments, FATA and FATB (Salas & Ohlrogge, 2002). These enzymes play a role in proper plant development and are involved in the supply of necessary saturated FAs for the synthesis of essential metabolites such as long-chain sphingolipid bases (Chen et al., 2008). In addition, thioesterases are key in oilseeds and play an essential role in determining the amount, length, and composition of fatty acids in the storage lipid pool (Voelker, 1996).

FATA and FATB differ in substrate specificity. FATA shows high affinity for monounsaturated iloyl-ACP. Two groups of FATB, FATB1 and FATB2, have been found. FATB1 is only present in species that accumulate short- or medium-chain FAs; meanwhile, FATB2 is widely distributed in the plant kingdom and is specific for palmitoyl and stearoyl-ACP (Aznar-Moreno et al., 2018).

When analyzing the sequenced region of the mRNA belonging to the FATA gene (NCBI accession number OL757543.1) using the BLAST tool, the alignment map observed in Figure 5B is obtained, where little alignment is observed in the initial region of approximately 200 bp.

Likewise, as shown in Figure 6B, this first segment was selected for further alignment analysis, resulting in a section for which no significant alignments were observed in species other than *T. alata*. However, when performing restriction site analysis with Benchling tool to ensure iGEM standards, the presence of a BsaI site is found in the selected region, therefore, a second selection of a shorter 133 nt fragment is performed.

For the mRNA sequence belonging to the FATB1 gene (NCBI accession number OL757544.1), using the BLAST tool, we obtained the results shown in Figure 5C, with little local alignment in the final region of approximately 100 bp.

Thus, the final 241 bp segment is selected, for which a local alignment map is obtained as shown in Figure 6C with a single coincidence in a 28 nt segment that is registered in the genome of the organism *Misopates orontium*, an organism whose presence in Colombia has not been reported and which, in addition to having a short alignment with the selected segment, results in a low probability of formation of significant amounts of siRNAs when producing multi potent and diverse siRNAs.

Finally, the alignment map is performed using the BLAST tool for the FATB2 mRNA, presenting very few matches in the initial segment of approximately 200 bp, as shown in Figure 5D. From there, the initial 250 bp segment is selected to perform a subsequent alignment analysis with BLAST as shown in Figure 6D, obtaining no significant similarity.

All the selected sequences can be accessed in [this link](#).

III. Expression vector: plasmid pET28a

The pET28a vector is one of the most popular expression plasmids on the market, useful to produce recombinant proteins in *Escherichia coli*. This vector belongs to the pET family of plasmids designed for T7 polymerase-mediated expression. Specifically, the pET28a plasmid contains the T7 promoter followed by the lacO operator and a ribosomal binding site (shine dalgarno sequence) and then, a multiple cloning site, with the presence of different restriction sites such as XhoI, NotI, HindIII, SalI, SacI, EcoRI, etc. In addition, it has the presence of a coding region for the Poly-Histidine purification tag (His6). The vector has the presence of a coding region for Aminoglycoside-3'-phosphotransferase (Kanamycin resistance marker), and the coding region for LacI, a repressor that binds to the LacO operator and is released in the presence of IPTG (Shilling et al., 2020).

- **T7 promoter:** As stated in iGEM's Registry of Standard Biological Parts, the T7 inducible promoter is useful for high expression levels in the presence of T7 polymerase, compatible with strains such as *E. coli* BL21 (Hausjell et al., 2020). It is useful for the expression of recombinant proteins and has been shown to be effective for the efficient production of recombinant RNAs such as siRNAs or dsRNAs (Donzé & Picard, 2002; Huang & Lieberman, 2013), by inducing high expression rates with the inducers IPTG and L-arabinose.

- **LacO:** This region represents a regulatory site located immediately after the promoter, it is an operator that facilitates the regulation of transcription, this region is repressed in the presence of the LacI repressor, that binds to the sequence preventing the action of RNA polymerase, however, in the presence of allolactose or IPTG this region is released and transcription is allowed when the polymerase binds to the promoter (Bell & Lewis, 2000).
- **P19_HisTag:** p19 protein is a protein expressed in *Thrombovirus* (Qiu et al., 2002), and plays a role in protecting the viral genome from siRNA-mediated degradation by conjugating to siRNAs and preventing the formation of RISC complexes (Danielson & Pezacki, 2013). This protein has been shown to be useful in siRNA expression systems by preventing siRNA degradation and facilitating purification (Huang et al., 2013b). On the other hand, the incorporation of polyhistidine (6 histidine residues) at the C' terminal end of the protein structure facilitates its purification by different techniques such as nickel-HPLC, thanks to the affinity of this polyhistidine chain with nickel beads.
- **hpRNA:** hpRNAs were designed in such a way that when expressed in *E. coli* and processed by RNase III enzymes, they form short siRNAs specific for the 4 target genes in *T. alata*. The fragment consists of three sections: a section of approximately 250 bp (or 133 in the case of the FATA gene) in forward sense, specific for the selected gene; a linker section, which will form the loop in the hpRNA and was designed according to Huang et al. (2013b), which incorporates several restriction sites, but at the same time is compatible with the RFC1000 standard as it does not contain restriction sites for Bsa1 and Sap1; and finally the reverse sequence of the first section, complementary to it in order to facilitate the formation of the hairpin structure.

The other parts of the vector were chosen to favor the standardization of the method and will be mentioned in the Standardization section.

IV. *E. coli* BL21 AI as chassis for siRNA production

The use of *E. coli* emerges as an alternative to the synthetic production of siRNAs, allowing a reduction in production costs, as well as the generation of multipotent siRNAs from 200 - 300 bp dsRNAs using its own enzymes (like DICER in eukaryotes) such as RNase III (Xiao et al., 2009). On the other hand, *E. coli* allows versatility in the design of the genetic circuit, as it presents a greater number of biological parts registered for the design and customization of the vectors. For example, a vector could be cotransformed for the expression of p19, a key protein in the stabilization of siRNAs, as well as the expression of T7 RNA polymerase, useful for expression as the specific T7 promoter is present (Huang et al., 2013b).

To ensure stability of the plasmids used, the *E. coli* BL21 AI strain, optimal for co-expression of siRNA and p19 protein, has been selected as a chassis for the purpose of the project.

E. coli BL21 AI strains are useful for the expression of recombinant proteins regulated under the T7 promoter, due to the presence of the chromosomal insertion for the T7 RNA polymerase cassette (T7RNAP) in the araB locus of the araBAD operon, thus generating a regulation of the production of this polymerase under the araBAD promoter, which is inducible in the presence of high concentrations of L-arabinose and repressed in the presence of high concentrations of glucose, allowing versatility in the expression of the recombinant product of interest, for example by allowing biomass growth without the production of T7RNAP and then promoting protein expression using the L-arabinose inducer (Thermo Fisher Scientific, 2015).

2.2.2. Standardization

For the construction of the genetic circuit, the iGEM Type IIS assembly method is followed, according to the RFC1000 standard, with the purpose of achieving the assembly of multiple scalable parts, allowing the development of more complex circuits in an efficient way.

To analyze compatibility with the RFC1000 standard guidelines, the presence of restriction sites for the Bsa1 and Sap1 enzymes was examined using the tool Benchling. Initially, the presence of the Bsa1 site was reported in the biological part designed for the construction of the 250 bp hpRNA specific for the FATA gene, however, a smaller fragment of 133 bp was selected according to the mentioned reference. On the other hand, the analysis of restriction sites for BglII and BlpI, essential to guarantee a correct final assembly in the pET28a expression plasmid, was also performed. The Benchling tool did not detect the presence of these sites in sites other than those designed.

I. Parts

For the design, 14 new parts were created and 3 existing iGEM parts were used, as well as 4 plasmids registered according to the standard assembly method used. 5 basic parts, 5 composite parts and 4 devices were designed, as follows:

- iDLBB_004340, iDLBB_004341, iDLBB_004342, iDLBB_004343 and iDLBB_004344 are basic parts, and refer to the individual sequences that are transcribed in the p19 protein with the polyhistidine tag or in each of the hpRNA segments of the FA-synthesis-related proteins. These parts fulfill the RFC1000 standard.

- iDLBB_004345, iDLBB_004346, iDLBB_004347, iDLBB_004348 and iDLBB_004349 are composite parts, and refer to the cassettes or transcriptional units (TU) of each of the basic parts of the circuit with the T7 promoter with the LacO operator, the T1 terminator and, in the case of p19, with the RBS. These parts fulfill the RFC1000 standard.
- iDLBB_004350, iDLBB_004351, iDLBB_004352 and iDLBB_004353 are devices, and refer to multitranscriptional units (MTU), consisting of the TU of the tagged p19 and the TU of one of the FA-synthesis-related protein hpRNAs. These parts fulfill the RFC1000 standard.

Figure 7 is a representation of the devices and their component parts, elaborated with SBOL Canvas, a computational tool that allows creating and editing genetic circuits under the SBOL visual standard. For more information on each of the parts used, go to the [following link](#).

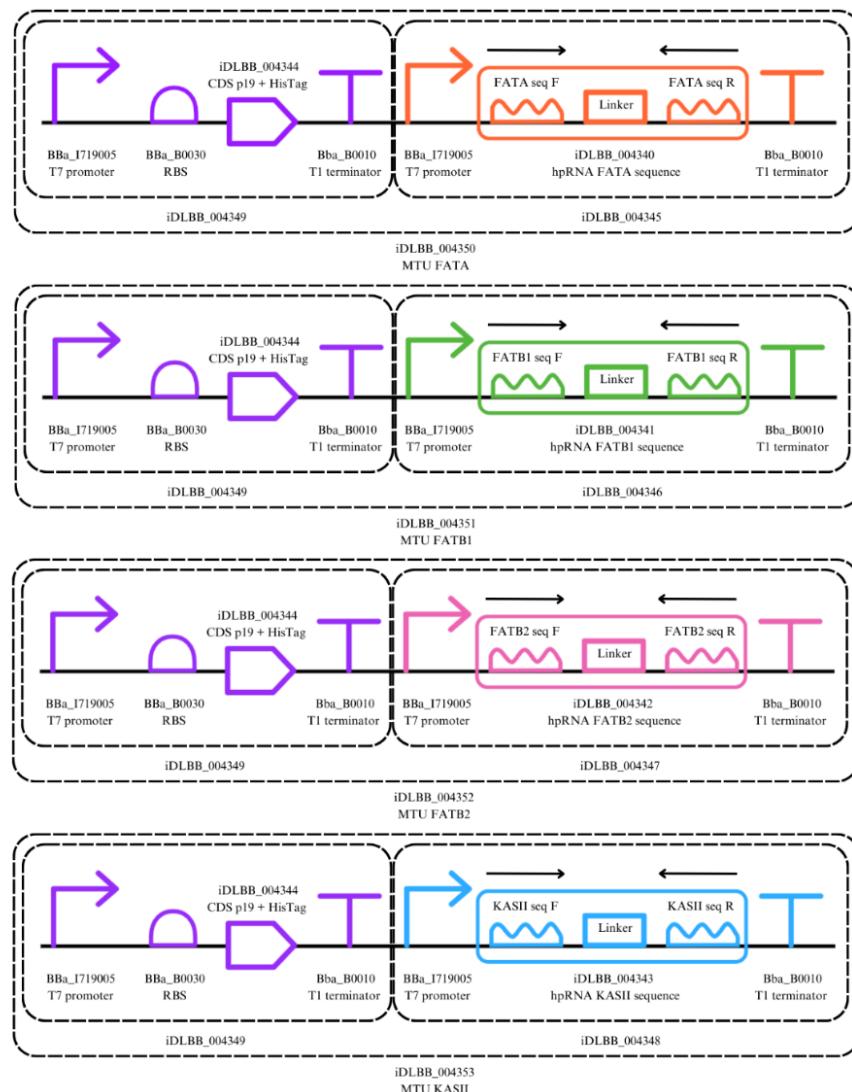


Figure 7. Representation of designed genetic parts using the SBOL visual standards.

II. Type IIS Assembly

For assembly, it is necessary to follow a series of steps to obtain multi-transcriptional units (MTU) from single parts.

Initially, assembly of each part is given to pSB1C00, the universal basic parts receptor plasmid (level 0). This requires the synthesis or amplification of the parts by PCR, using prefixes and suffixes that include: Bsa1 fusion sites, for subsequent assembly of these into level 1 transcriptional units; Sap1 restriction sites, for correct assembly of each basic part to pSB1C00 by the Sap1 enzyme; bglII and BlpI fusion sites, for the correct assembly into the final expression vector; and the typical SCARS or fusion sites for the basic parts according to the RFC1000 standard.



Figure 8. Representation of basic biological part of T7 promoter with prefix and suffix

For example, Figure 8 shows the T7 promoter with its corresponding prefixes and suffixes, and the other basic parts were constructed in the same way, each of which was inserted in the level 0, just as some of them are shown in Figure 9.

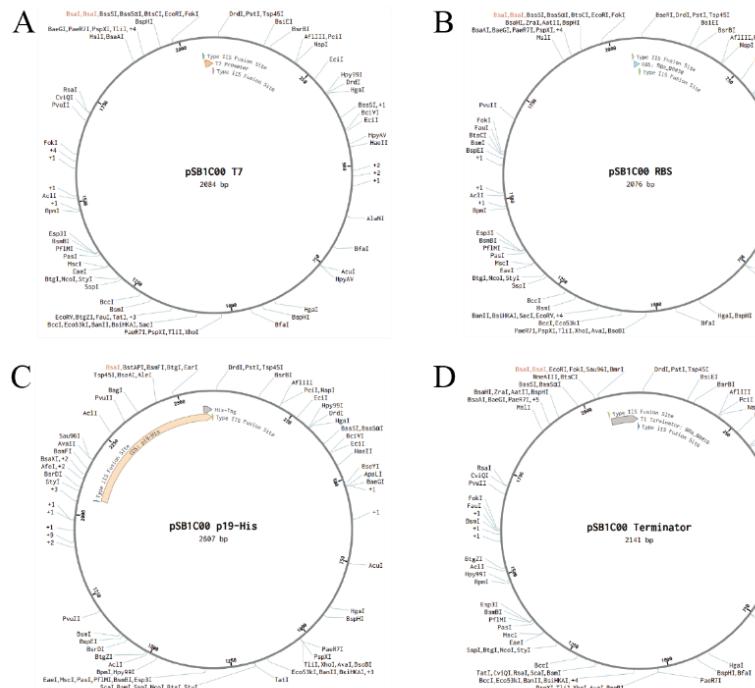


Figure 9. Level 0 vectors for basic parts assembly

Having the individual parts assembled in pSB1C00, it is possible to digest them using the enzyme BsaI to assemble them into level 1 transcriptional units (TU). When working with 5 sets of cassettes (1 for tagged p19 protein and 4 others for each target gene), it is necessary to assemble them like that: into the pSB1K01 level 1 vector for getting the p19 protein TU, and into pSB1K02 level 1 vectors for the hpRNAs TUs, so that when they are digested with the Sap1 enzyme, the fusion of each TU is correctly performed in the desired order and with the specific scars. For instance, Figure 10 shows the assembly process of each basic unit for the formation of the p19 TU in the pSB1K01 vector.

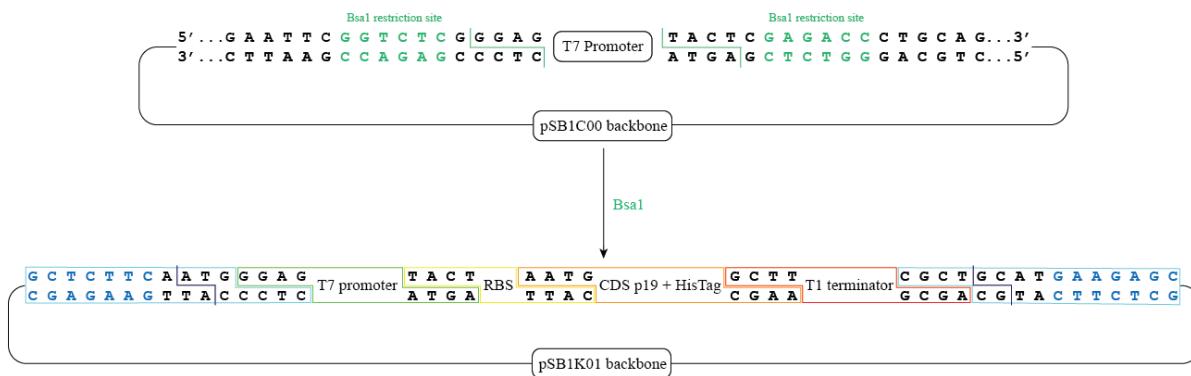


Figure 10. Assembly process of p19 TU from basic parts

Again, following the same process for each of the basic parts, the TUs were inserted into level 1 plasmids as described above, obtaining 5 structures like those shown in Figure 11.

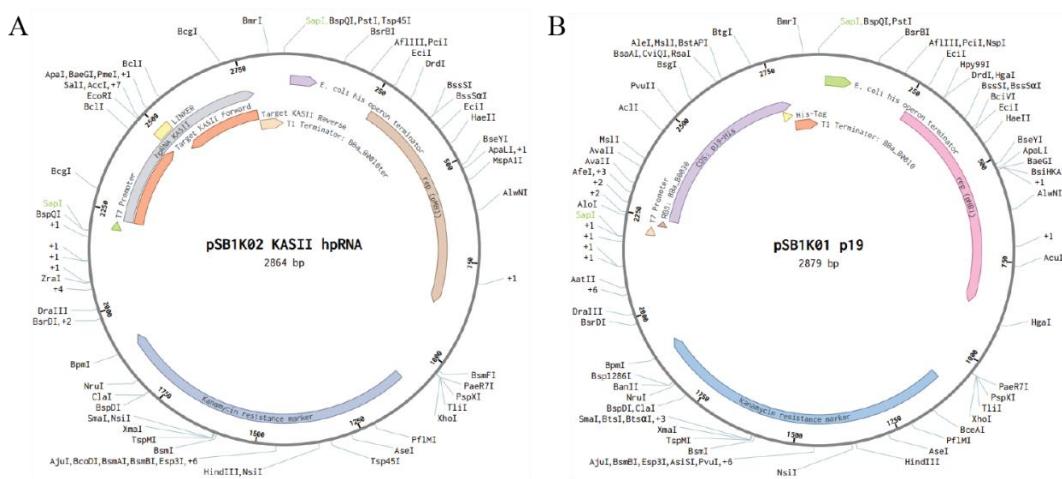


Figure 11. Level 1 vectors for p19 and KASII hpRNA TU assemblies

Finally, a multitranscriptional unit (MTU) is obtained by assembling the TUs into level 2 vector pSB3C11, through the action of the Sap1 enzyme on the level 1 vectors. In this way, 4 genetic circuits composed of two TUs each one (tagged p19 protein and hpRNA) are assembled. In addition, by presenting the MTU flanked between Bsa1 restriction sites, the construction of even more complex genetic circuits is allowed by digesting the plasmid with the respective Bsa1 restriction enzyme and incorporating MTUs into level 3 vectors. Figure 12 shows the assemblies of the genetic circuits.

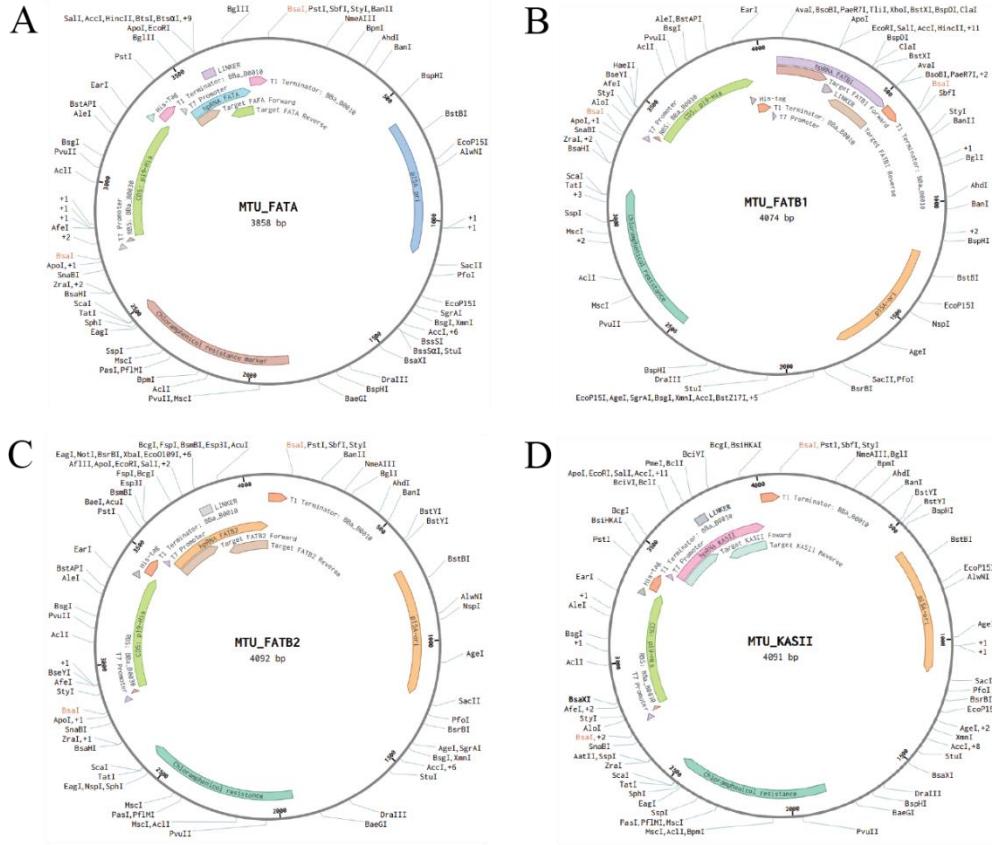


Figure 12. Assemblies of MTUs in level 2 vectors

pSB3C11 vector, reported in iGEM's Registry of Standard Biological Parts, is a level 2 pEven1-type plasmid, useful in the construction of genetic circuits with multitranscriptional units (MTU), and created by PCR mutagenesis of the pSB3C01 plasmid, for the removal of the illegal Bsa1 site, allowing the assembly of MTU assemblies by the iGem Type IIS assembly IIS method. The vector incorporates a chloramphenicol resistance marker in its sequence, which will allow the selection of transformed *E. coli* strains.

On the other hand, no evidence is reported about the recombinant protein expression rates of this vector, so further evolution of techniques such as Red-mediated recombination system is proposed, for the insertion of MUT at chromosomal level in *E. coli*, as demonstrated by Nakamura et al. (2018), obtaining high protein expression rates in *E. coli* by insertion of transcriptional units with the T7 promoter. However, an expression vector such as pET28a could be used to express the MTUs, The Level 2 plasmids can be digested with the enzymes BglII and BlpI as well as the expresion plasmid, in order to obtain a construct with high levels of expression of p19 and the hpRNAs.

2.2.3. Genetic Circuits

In the design of the genetic circuit, the relationships between the different standardized biological parts and the biological parts present in both the chassis, *E. coli*, and the plant cells of *T. alata* are analyzed. Initially, when selecting the BL21 AI strain of *E. coli*, the genomic cassette for the expression of T7 RNA polymerase inserted in the araB locus, regulated under the araBAD promoter, which presents regulation, being inducible in the presence of L-arabinose.

On the other hand, the design of the plasmid vector makes use of the T7 promoter, which can be recognized only by the T7 polymerase, and, being bound to the lac operator (lacO), is inducible in the presence of isopropyl- β -D-1-thiogalactopyranoside (IPTG). IPTG is a non-metabolizable compound that is not broken down by *E. coli*, making it an ideal reagent; furthermore, it is a molecular analog of allolactose, which removes the repressor lacI from the lacO to induce gene expression.

This promoter will be in charge of regulating the expression of two fundamental biological parts in the circuit design: it regulates the expression of the p19 protein tagged with polyhistidines (HisTag) and with a commonly used RBS, while it regulates the production of specific hpRNAs for the target genes, composed of forward and reverse sections, in addition to the linker segment, essential for the formation of the hairpin loop. Likewise, the use of the T1 terminator is made for both parts, efficient for the termination of the transcription process in different cloning vectors for *E. coli*.

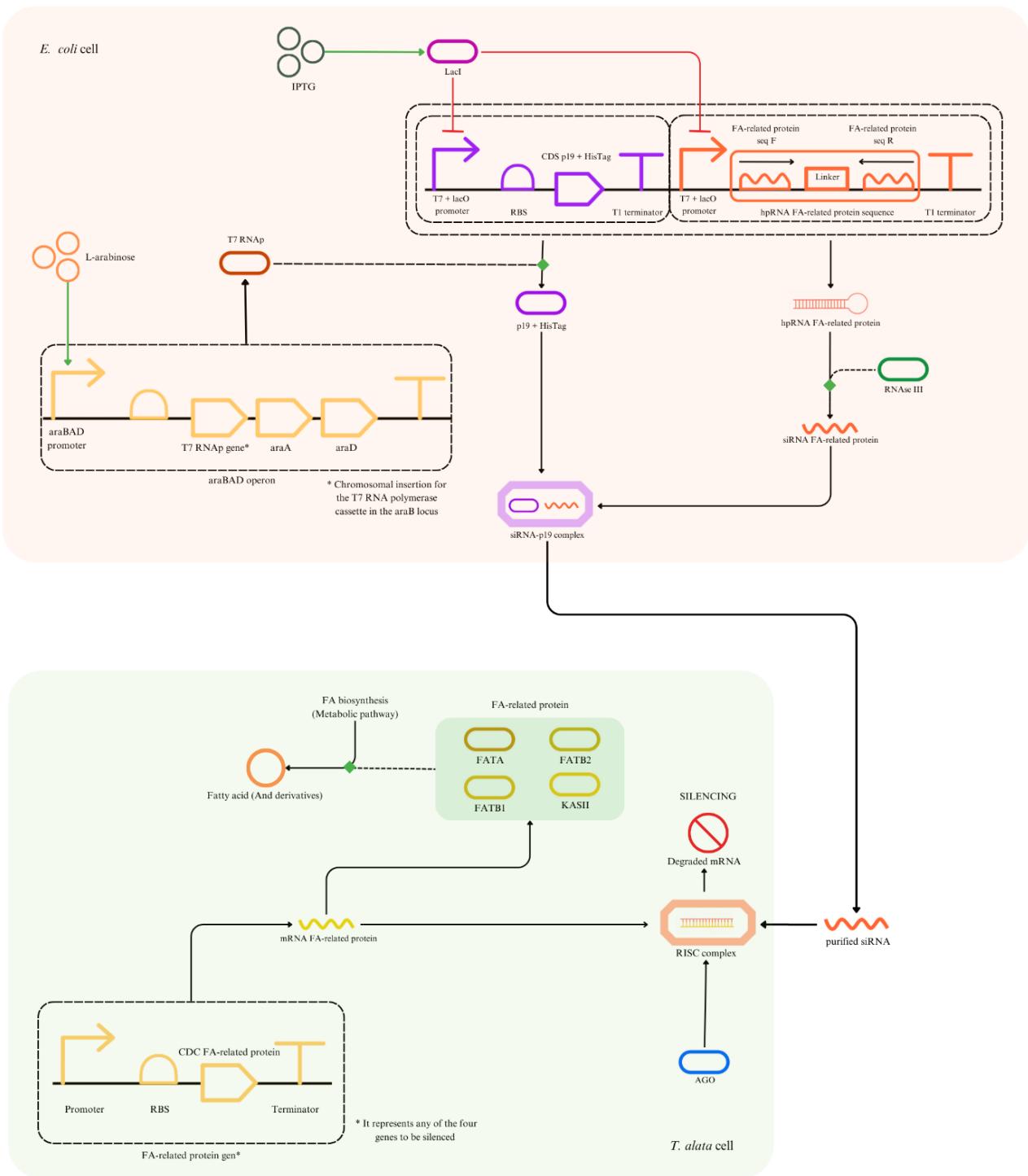


Figure 13. Designed genetic circuit under the SBOL visual standard

From MTUs, hpRNAs will be produced, which will be processed by RNAase III enzymes in *E. coli*, leading to the formation of specific siRNAs for the target genes. It is here where the p19 protein is essential, which binds to the siRNAs, forming the siRNA-p19 complex to prevent their degradation and

preserve their integrity. Subsequently, the siRNAs are purified by releasing them from the complex and extracting them from *E. coli*.

When the siRNAs are incorporated into *T. alata* cells, they are recognized by the argonaute proteins (AGO), leading to the formation of the RISC silencing complex, which recognizes the mRNAs produced from the transcription of the target genes for which there are promoters, RBS, CDS and terminators specific to this organism. The RISC complex gives way to the degradation of the mRNAs by excision, thus silencing the protein expression of each fatty acid-related target gene, disrupting steps in the metabolism of fatty acids and their derivatives (see "Abstraction" for an understanding of the metabolic effect of silencing).

2.3. Excellence in Biological Engineering

2.3.1. Optimization

I. Optimization of model specificity and standardization: BLAST and restriction mapping with Benchling

To ensure the selectivity of the gene silencing method used, a local alignment of the selected target genes is performed to find unique regions in the species using the BLAST tool. From this analysis, sequences of approximately 250 bp are selected from which another local alignment is performed to corroborate the selectivity of the chosen sequences, in addition to a restriction analysis using the Benchling tool to ensure that no BgIII, BlpI, Bsa1 and Sap1 restriction sites are present, which will be relevant when assembling the parts and the construct in the expression vector. According to the findings, sequences of 250 nt for the KASII and FATB1 genes, 133 nt for FATA and 144 nt for FATB2, which comply with the established conditions, were finally selected.

II. Optimization of Target-Linker-Target sequences for hairpin structures formation

After selecting the specific and selective sequences (forward segment) for the target genes by local alignment (BLAST), the reverse sequence is obtained using the Reverse complement tool of the Sequence Manipulation Suite software by GenScript Biotech Corporation and this sequence is incorporated into the reverse sequence, intersected by a linker segment selected as reported by Huang et al. (2013b).

Then the prediction of RNA secondary structures is performed in the [RNAfold web server software of the Institute for Theoretical Chemistry of the University of Vienna](#). The structures shown in Figure 14 are obtained, where the formation of Hairpin-type RNA secondary structures can be evidenced.

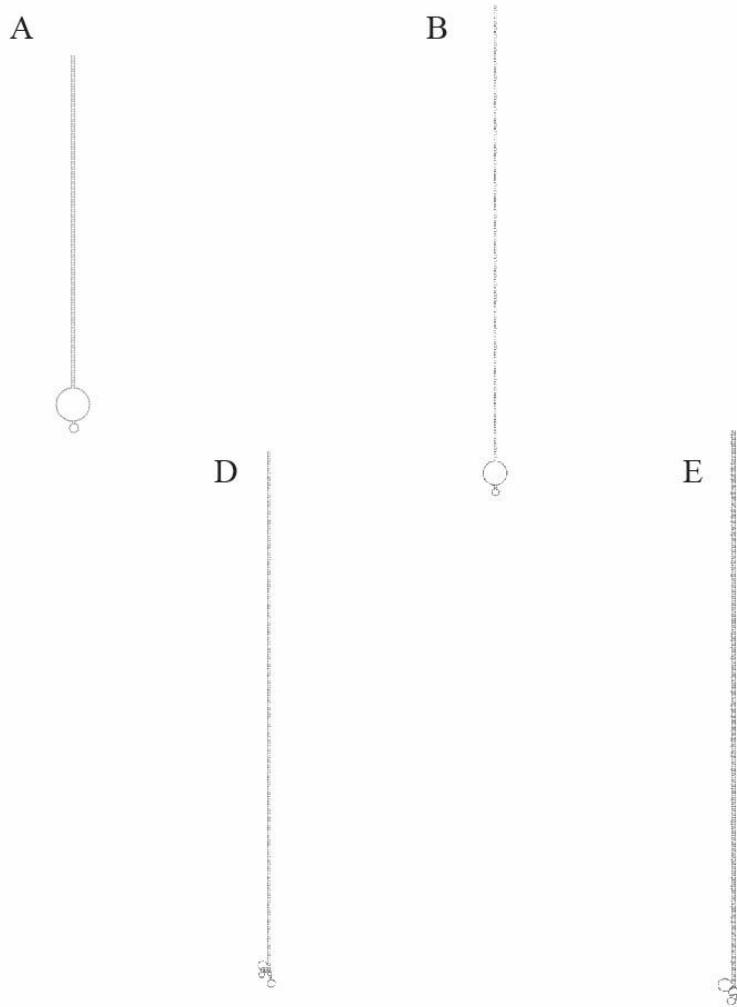


Figure 14. Simulated hairpin structures for: (A) FATA, (B) FATBI, (C) FATBII, (D) KASII hpRNAs

III. Prediction of generated siRNAs: Thermofisher RNAi designer

As mentioned in the design of the genetic circuit, hpRNAs will be produced and processed by the enzyme RNase III to produce multipotent siRNAs, which will have different sequences in the selected range for the target genes FATA, FATB1, FATB2 and KASII. The [BLOCK-iT™ RNAi Designer tool from Thermo Fisher Scientific](#) is used to design effective siRNAs, and the possible formation of diverse siRNAs with high probability of effectiveness for gene silencing is appreciated. The tool reports 10

possible siRNA structures for each target gene with optimal scoring according to the tool parameters, and filtered by G/C percentages between 35-55 %. You can find the predicted siRNAs in [this link](#).

IV. Modeling of p19 protein: protein - siRNA interaction

A fundamental concern for the construction of the circuit is to guarantee the stability and avoid degradation of the siRNAs, as well as to facilitate their purification, processes for which His-tagged p19 protein plays an essential role. It is therefore necessary to understand the structure, function, and characteristics of the protein in order to optimize the conditions for its use in the Wet-Lab.

Then, a modeling of the tertiary structure of the protein was performed in the computational biological tool [Swiss-Model](#), from the amino acid sequence in FASTA format registered under the accession number WAK97598.1 in the NCBI protein database, obtaining as a result the model predicted in Figure 15, where the concavity where the siRNA binds is observed.

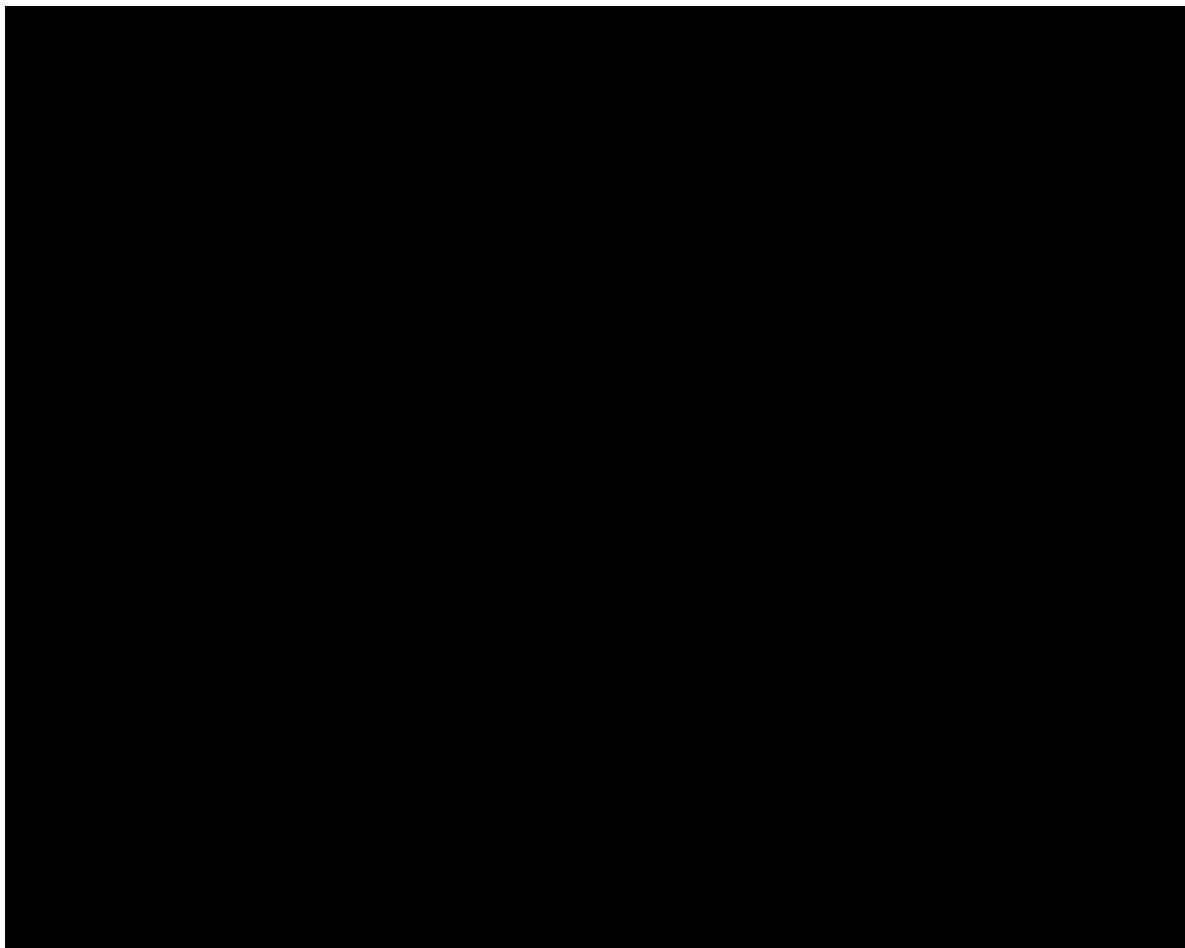


Figure 15. 3D model of p19 protein tertiary structure, made in Swiss-Model

Subsequently, this amino acid sequence was given into Expasy's ProtParam, a tool that allows the computation of several physical or chemical parameters of a given protein, to find the theoretical parameters for the characterization and best performance of the molecule, as well as the estimation of its half-life in the used chassis. The results are reported in Table 1.

Table 1. Theoretical parameters of His-tagged p19 protein obtained in ProtParam

Parameter	Value
Number of amino acids	181
Molecular weight	20449.32
Theoretical pI	5.84
Number of negatively charged residues	25
Number of positively charged residues	19
Extinction coefficients	33585 (Abs 0.1 % (= 1 g/L: 1.642, assuming all pairs of Cys form cystines) 33460 (Abs 0.1 % (= 1 g/L: 1.636, assuming all Cys are reduced)
Estimated half life	> 10 h (<i>E. coli</i> , <i>in vivo</i>)
Instability index	53.37 (Unstable)
Aliphatic index	53.31
Grand average of hydropathicity	-0.854

Although most parameters provide a physicochemical characterization of the protein, it should be noted that its half-life in *E. coli* is desirable, at least for a first stage of construction; however, the molecule was classified as unsTable , so, in order to optimize the model, the use of the IPTG and L-arabinose T7 promoter's inducers is proposed to induce high rates of transcription and, consequently, translation of the protein, increasing its concentration and improving its long-term stability.

On the other hand, the Swiss-Model tool makes use of pre-existing templates modeled by other authors for protein modeling, and allows the use of these; so, when modeling the protein, it was possible to find a template of the interaction model between the protein and the siRNA, modeled with x-ray diffraction method by Foss et al. (2019), which shows how the polypeptide sequesters the RNA molecule (Figure 16), forming a complex that protects it from degradation and allowing its purification, as expected in the designed model.

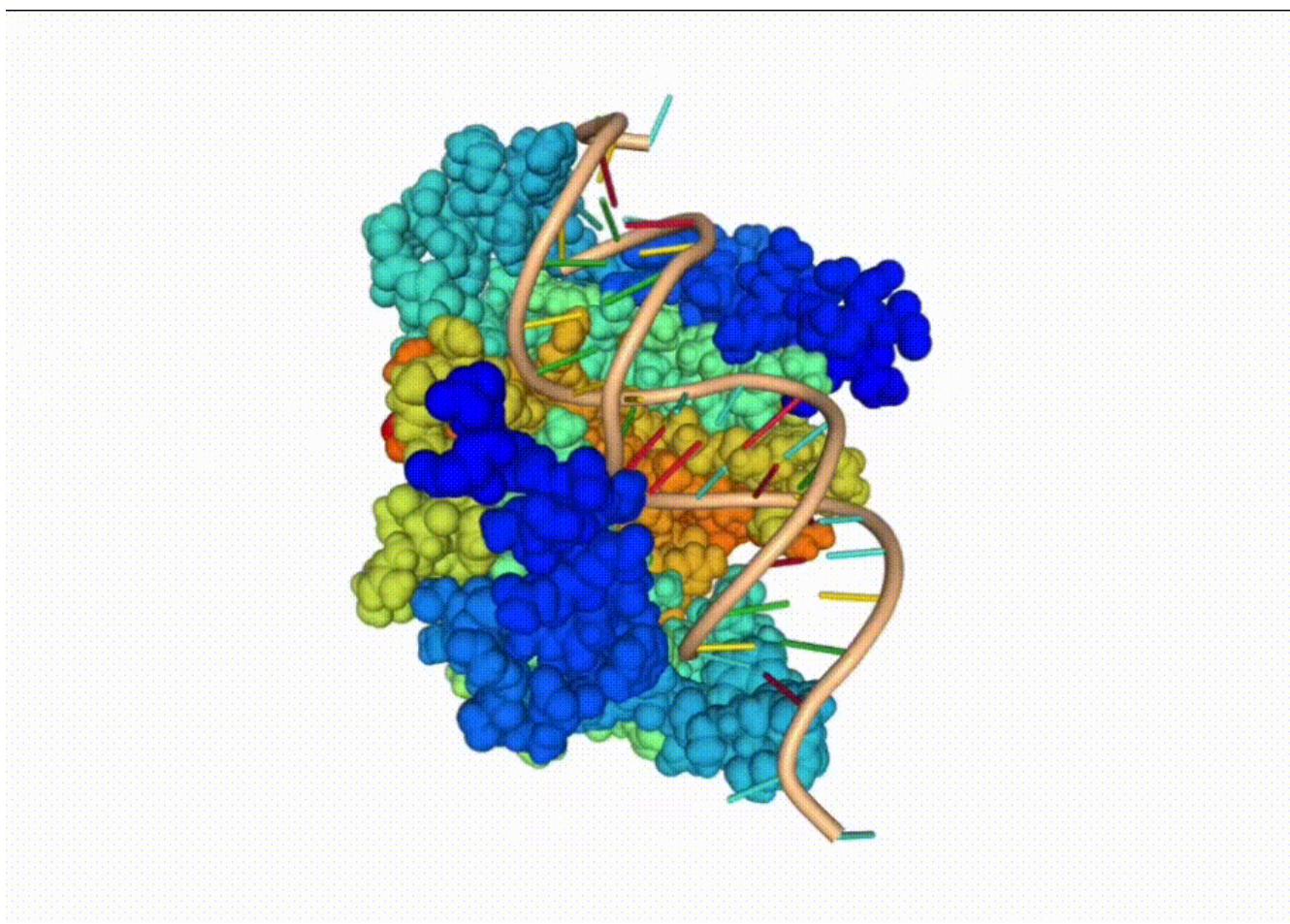


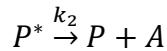
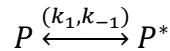
Figure 16. p19 protein in complex with siRNA (Foss et al., 2019)

V. Mathematical modeling

For all the proposed mathematical models some approximations were made to simplify the system design, besides not taking into account the possible noise in the system (it approximates a deterministic model). The first is the use of only the promoter to represent the complete transcriptional unit; the second is, for the p19 protein expression model, to ignore transcription, treating translation as a single-step event; finally, a third approach, it is decided to omit RNA polymerase (RNAP), as its concentration is higher than that of the promoter and is assumed constant during the interaction with the transcriptional units. Even in the modeling that takes into account the induction process, the regulation by L-arabinose is ignored, since it is indirect and the concentration is assumed to be at saturation, i.e., maximum expression of T7 RNAP.

a. hpRNA and His-tag p19 protein expression levels: Simple model

In a first step, the objective is to obtain an estimation of the concentrations of the species in steady state (equilibrium) through mass action kinetics assuming the highest activity of the promoters, without taking into account their induction systems, in one E. coli cell. The following biochemical reactions were developed:



Where P is the inactive promoter, P* is the active promoter (the polymerase binding is implicitly taken into account) and A is the product, either hpRNA or p19 protein, generalized to arrive at a common expression, taking into account the similarity of their transcriptional units. In turn, k1, k-1, and k2 are the rate constants of the reactions and kd the rate constant of degradation of the product. The first reaction represents the binding of the transcriptional machinery to the promoter and its activation, the second the process of transcription (and translation for p19) as such, and the third the degradation of the product.

Now, from the reaction rates the following system of ordinary differential equations (ODEs) can be derived:

$$\frac{d[P]}{dt} = -k_1[P] + (k_{-1} + k_2)[P^*]$$

$$\frac{d[P^*]}{dt} = k_1[P] - (k_{-1} + k_2)[P^*]$$

$$\frac{d[A]}{dt} = k_2[P^*] - k_d[A]$$

Now, at steady state, there is no change in the concentration of any of the species, so the ODEs can be rewritten as follows:

$$-k_1[P] + (k_{-1} + k_2)[P^*] = 0$$

$$k_1[P] - (k_{-1} + k_2)[P^*] = 0$$

$$k_2[P^*] - k_d[A] = 0$$

The matrix of stoichiometric coefficients N of the above system of equations is:

$$N = \begin{pmatrix} -1 & 1 & 1 & 0 \\ 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix}$$

From which it can be observed that the first row is equal to the second row multiplied by -1, i.e., there are two linearly independent rows, so the number of conserved quantities Q is 1, so that equation can be replaced by a conservation relation. To realize the above, it can be stated that the total promoter concentration is conserved:

$$[P_T] = [P] + [P^*]$$

So it can be replaced in the system of equations, as follows:

$$[P_T] = [P] + [P^*]$$

$$k_1[P] - (k_{-1} + k_2)[P^*] = 0$$

$$k_2[P^*] - k_d[A] = 0$$

From what is obtained:

$$[P^*] = \frac{[P_T]}{1 + \frac{k_{-1} + k_2}{k_1}}$$

$$[P] = [[P_T] - [P^*]]$$

$$[A] = \frac{k_2[P^*]}{k_d}$$

In the absence of such specifications for the parties involved, the standard parameters reported by Marchisio & Stelling (2008) and the degradation rates reported in , adapted in Table 2, are used to estimate species concentrations.

Table 2. Standard parameters for a basic promoter system

Parameter	Value	Unit
P_T	2.8×10^{-8}	M
k_1	10^6	$M^{-1}s^{-1}$
k_{-1}	0.01	s^{-1}
k_2	0.03	s^{-1}
$k_{d,hpRNA}$	8.05×10^{-6}	s^{-1}
$k_{d,p19}$	0.00116	s^{-1}

Thus, the following estimates are obtained:

$$[P^*] = 2.8 \times 10^{-8} M$$

$$[P] = 0 M$$

$$[hpRNA] = 1.044 \times 10^{-4} M$$

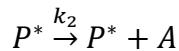
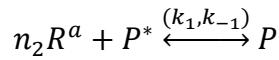
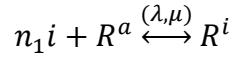
$$[p19] = 7.241 \times 10^{-7} M$$

Note that the promoter is estimated to be fully active. The problem with the above model is that it does not take into account that the T7 promoter, due to the presence of lacO, is inducible and its activation level depends on the concentration of the inducer, IPTG.

b. hpRNA and His-tag p19 protein expression levels: Induced model

Now, to fit the model to the proposed genetic circuit, we use kinetics based on Hill functions for the characterization of regulated promoters, for which the Hill coefficient or cooperativity index, n, and the

Hill constant, K_H , must be estimated. Let R be the lacI repressor and i the IPTG inducer, ignoring their production and degradation, the proposed reactions are as follows:



Where A still represents any of the products to be estimated (hpRNA, p19); P the inactive promoter and P^* the active promoter; R^a and R^i , the active and inactive repressor by action of the inducer, respectively; and λ and μ the rate constants of association and dissociation of the inducer to the repressor, respectively. The first reaction illustrates the inactivation of the repressor by the inducer; the second the inactivation of the promoter by the repressor; the third is the production of A from the active promoter; and the last is the degradation reaction of the A product.

The species dynamics can be written as follows:

$$\frac{d[P]}{dt} = k_1[P^*][R^a]^{n_2} - (k_{-1})[P]$$

$$\frac{d[P^*]}{dt} = -k_1[P^*][R^a]^{n_2} + (k_{-1})[P]$$

$$\frac{d[A]}{dt} = k_2[P^*] - k_d[A]$$

$$\frac{d[R^a]}{dt} = -\lambda[R^a][i]^{n_1} + \mu[R^i] - k_1[P^*][R^a]^{n_2} + (k_{-1})[P]$$

$$\frac{d[R^i]}{dt} = \lambda[R^a][i]^{n_1} - \mu[R^i]$$

$$\frac{d[i]}{dt} = -\lambda[R^a][i]^{n_1} + \mu[R^i]$$

In addition, the total promoter concentration can also be considered as a conserved amount:

$$[P_T] = [P] + [P^*]$$

With this, and considering the Hill constant KH raised to the coefficient n, for an inverse reaction, is equal to the quotient between the rate constant of the reaction between the rate constant of the inverse reaction, of the dynamics of P* in equilibrium (without change in its concentration in time) its concentration can be written as follows:

$$[P^*] = \frac{1}{k_{-1} [R^a]^{n_2} + 1} [P_T] = \frac{1}{(\frac{[R^a]}{K_H})^{n_2} + 1} [P_T]$$

Introducing the above expression in the dynamics of the product A, and knowing that, in general, k2[PT] is considered a different rate constant, ktr, the rate of change of the product over time can be expressed as follows:

$$\frac{d[A]}{dt} = k_{tr} \frac{1}{(\frac{[R^a]}{K_H})^{n_2} + 1} - k_d [A]$$

The total amount of free repressor can be assumed to be another quantity conserved at equilibrium:

$$R_f = R^a + R^i$$

From which the steady-state concentration of the activated repressor can be written as follows, differentiating the new Hill constant with the notation Kh:

$$[R^a] = \frac{1}{\frac{\lambda}{\mu} [i]^{n_1} + 1} [R_f] = \frac{1}{(\frac{[i]}{K_h})^{n_1} + 1} [R_f]$$

If this equation is substituted into the expression describing the concentration of the active promoter found above, we obtain:

$$[P^*] = \frac{[P_T]}{\frac{[R_f]^{n_2}}{K_H^{n_2} (1 + \frac{[i]^{n_1}}{K_h^{n_1}})^{n_2}} + 1}$$

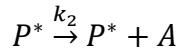
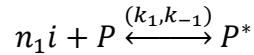
Now, with this new expression in the dynamics obtained for the product A, the ODE corresponding to the dynamics of A is obtained:

$$\frac{d[A]}{dt} = k_{tr} \frac{1}{\frac{[R_f]^{n_2}}{K_H^{n_2}(1 + \frac{[i]^{n_1}}{K_h^{n_1}})^{n_2}} + 1} - k_d[A]$$

It is noteworthy how, under this new model, the concentration of the desired product depends on the concentration of inducer i (IPTG). However, the resulting equation is complicated and, as the product does not depend on the activated repressor, its dynamics can be rewritten through the general ODE deduced from the Hill functions for activated promoters () with a logic similar to the one used, where the inducer can be considered the activator:

$$\frac{d[A]}{dt} = k_{lk} + k_{tr} \frac{\left(\frac{[i]}{K_H}\right)^{n_1}}{\left(\frac{[i]}{K_H}\right)^{n_1} + 1} - k_d[A]$$

This model takes into account the rate constant k_{lk} of A production by promoter leakage caused by the binding of pRNA to inactive P promoters at low frequency, and the biochemical reactions on which it is based are as follows:



With the above in mind, in steady state, when the concentration of A does not change over time, and again without having the specific parameters for the parts involved, an estimation of the concentrations of hpRNA and p19 produced is made, using the standard parameters reported by Marchisio & Stelling (2008) for an inducible one operator promoter and the concentration of IPTG recommended for strain E. coli BL21 AI in Thermo Fisher Scientific, (2015), adapted in Table 3, and the degradation rates reported in Table 2, in the following equation:

$$[A] = \frac{k_{lk} + k_{tr} \frac{(\frac{[i]}{K_H})^{n_1}}{(\frac{[i]}{K_H})^{n_1} + 1}}{k_d}$$

Table 3. Standard parameters for an inducible one-operator promoter system

Parameter	Value	Unit
k_{lk}	10^{-5}	s^{-1}
k_2	0.03	s^{-1}
$[P_T]$	2.1×10^{-5}	M
k_{tr}	6.3×10^{-10}	$M s^{-1}$
λ	8.3×10^5	$M^{-1} s^{-1}$
μ	0.0017	s^{-1}
K_H	4.882×10^8	M^{-1}
n	1	-
$[i]$	0.001	M

The following estimates were obtained:

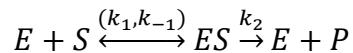
$$[hpRNA] = 1.2422 M$$

$$[p19] = 8.6207 \times 10^{-3} M$$

When comparing the estimates with the values obtained without taking induction into account, it can be concluded that there is a higher expression of the chemical species of interest under an induced system, this for one cell.

c. From hpRNA to siRNA: a Michaelis-Menten kinetics approximation

The cleavage of hpRNA to produce siRNAs is an enzymatic reaction catalyzed by the RNase III enzyme, so its behavior can be modeled through Michaelis-Menten kinetics. The Michaelis-Menten interaction scheme used is:



Where E refers to the RNase III enzyme, S is the substrate (hpRNA), ES is the enzyme-substrate complex and P is the product (siRNA). The rate of this writing is defined as:

$$\nu = \frac{d[P]}{dt} = k_2[ES]$$

Under the quasi-steady-state condition, the concentration of the ES complex changes slowly in time, so it can be treated as if it were in equilibrium and leads to the following expression:

$$0 = \frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES]$$

Under the assumption that the total enzyme concentration remains constant over time:

$$[E_T] = [E] + [ES]$$

Obtaining, with the definition of the Michaelis-Menten constant KM:

$$[ES] = \frac{k_1[E_T][S]}{k_{-1} + k_2 + k_1[S]} = \frac{[E_T][S]}{[S] + K_M}$$

Thus, the reaction rate is given by:

$$\nu = \frac{d[P]}{dt} = k_2[E_T] \frac{[S]}{[S] + K_M} = \nu_{max} \frac{[S]}{[S] + K_M}$$

Where the maximum reaction rate v_{max} is the product of the rate constant k_2 times the total enzyme concentration. However, to be more precise, the equation should consider the degradation of siRNAs:



The following reaction rate was obtained:

$$\nu = \frac{d[P]}{dt} = v_{max} \frac{[S]}{[S] + K_M} - k_d [P]$$

Thus, in steady state, where the change in product concentration over time is 0:

$$[P] = \frac{v_{max} \frac{[S]}{[S] + K_M}}{k_d}$$

With the substrate concentration (hpRNAs) obtained in the induced model, and with the kinetic parameters reported in for RNase III and in for the degradation rate of siRNAs, recorded in Table 4, the estimate of the concentration of siRNAs generated is:

$$[siRNA] = 2.3975 \times 10^3 M$$

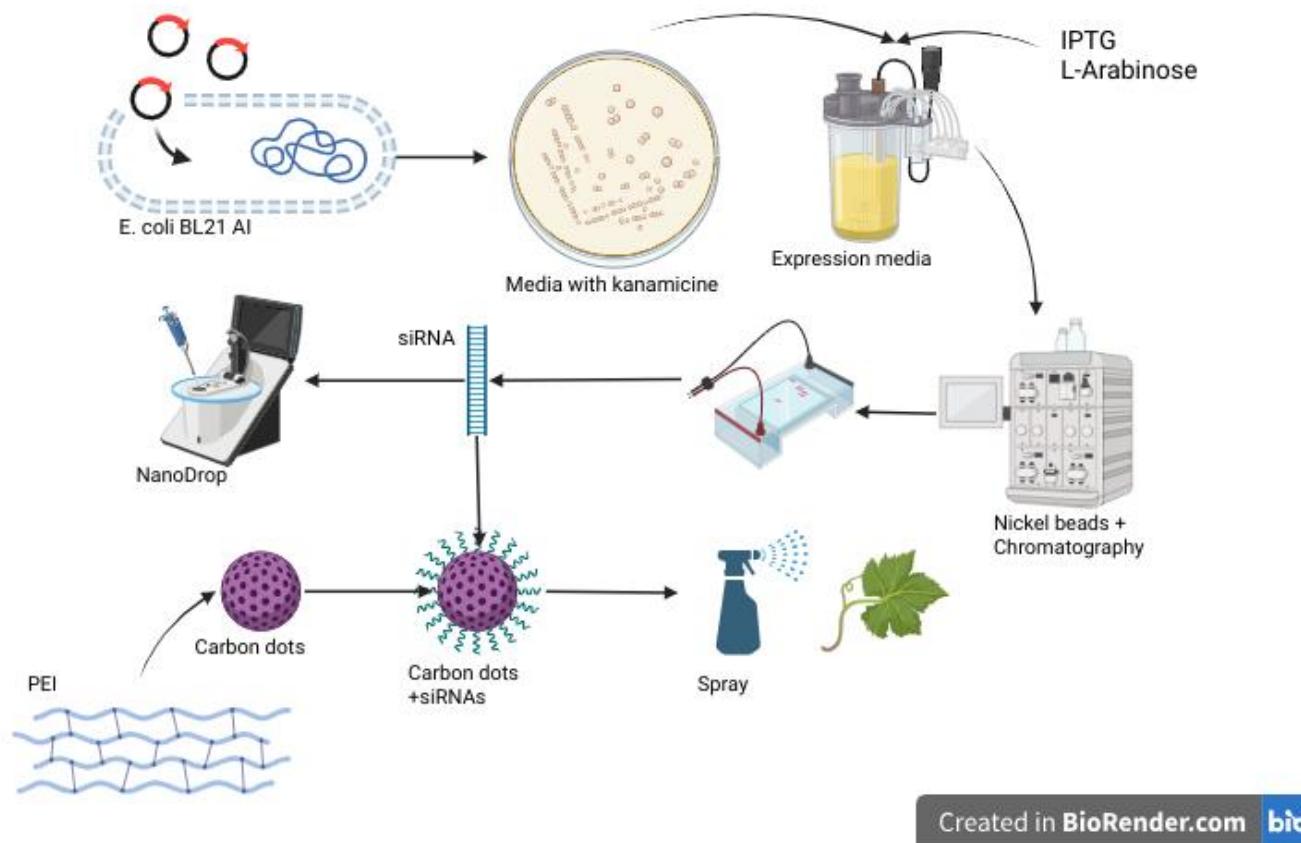
Table 4. Reported parameters for RNase III and degradation rate for siRNAs

Parameter	Value	Unit
K_M	4.2×10^{-8}	M
v_{max}	0.0193	s^{-1}
$k_{d,siRNA}$	8.05×10^{-6}	s^{-1}

2.3.2. Build and Test

Detailed build and test protocols can be found in [this folder](#).

2.3.2.1. Build



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Figure 17. Build protocols' graphic summary

I. Transformation procedure

For the transformation of the *E. coli* BL21-AI strain, a heat shock procedure is performed. Initially, the vial with the strain should be placed on ice, and then 10 ng of plasmid DNA in a volume of 5 μ L should be added to the cells and mixed by gently tapping the vial, then, it should be left to incubate on ice for 30 min and then the heat shock should be applied by incubating the vials in a water bath at 42°C for 30 s without shaking. After applying the heat shock, the vials are returned to ice and 250 μ L of SOC medium is added at room temperature. The vials should be secured in a microcentrifuge rack with adhesive tape and placed in an incubator with agitation at 37 °C for 1 h at 225 rpm. Finally, seeding is performed with selection by Kanamycin antibiotic in LB media to leave them in incubation at 37°C.

II. Modulating gene expression: p19-His, hpRNA and siRNA production

To modulate the expression of both p19-His and hpRNAs, a procedure is followed in which a significant amount of biomass is initially produced to induce the expression of the compounds of interest. Initially, 4-5 transformants are selected for cultivation in LB liquid medium supplemented with kanamycin in incubation at 37 °C and with agitation, measuring optical density until reaching an OD600 value between 0.6 and 1, cultures from which the most viable will be selected for a second inoculation into fresh medium supplemented with the antibiotic up to an optical density of 0.4 (2-3 hours of culture). Once at this point (mid-log phase), the induction of expression is performed by the addition of L-arabinose at a final concentration of 0.2% and IPTG at a final concentration of 1 mM

III. siRNA purification

Once the siRNAs have been expressed in *E. coli*, a protocol for their extraction and purification will be followed. Initially, cell lysis is performed using lysozyme and lysis buffer, followed by purification of the siRNAs using nickel beads (which, being affine to the poly-Histidine segments, retain these molecules), followed by their elution with SDS and finally purification by ion exchange HPLC. By analyzing the HPLC fractions by polyacrylamide gel electrophoresis, it is possible to select those segments with a chain length of approximately 21 nt corresponding to the siRNAs of interest. Finally, these are precipitated in isopropanol solutions and the pellet obtained is washed with 70% ethanol; they are dried and resuspended in nuclease-free water to measure the concentration of siRNAs obtained by spectrophotometry with NanoDrop.

IV. Carbon dots production, functionalization and siRNA aggregation

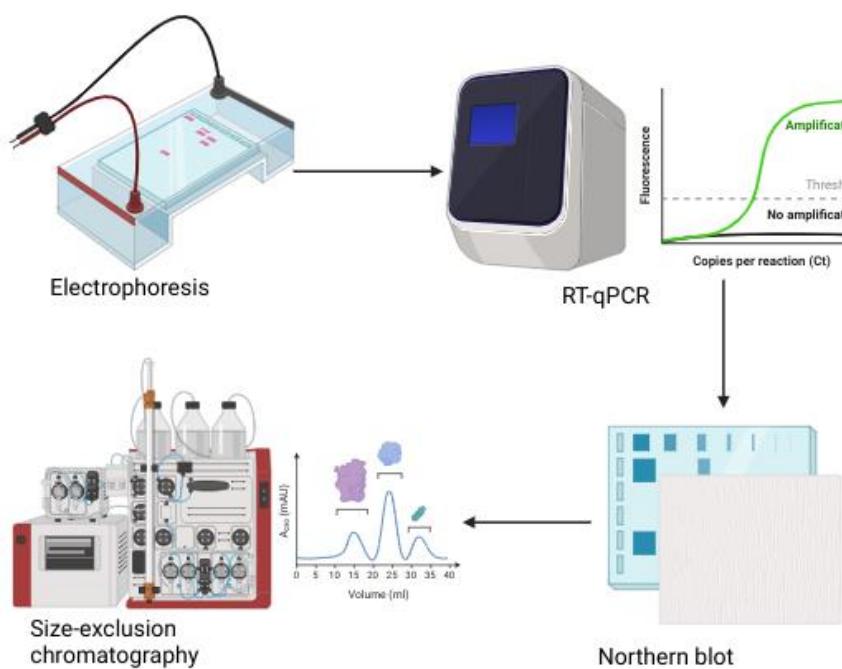
As proposed by Schwartz et al. (2020), carbon dots will be used for the delivery of siRNAs, in this procedure the production of carbon dots from polyethylenimine (PEI) is proposed, which will allow the formation of nanostructures of 2 - 10 nm in size, positively charged thanks to the presence of the amino groups of PEI, which would facilitate the binding of siRNAs to them, thus allowing their transport through the cell wall of plant cells and other consecutive membranes. A simple bottom-up method will be followed, which consists in the direct production of carbon dots from PEI, where PEI solutions of different molecular weights are heated at 155 °C in a mixture of chloroform and methanol for a short period of time, using a microwave synthesizer; then the chloroform is separated by centrifugation, dried with nitrogen and resuspended in water. Finally, the pH is adjusted to 8 and the carbon dots are purified by size exclusion chromatography equipped with spectrophotometry.

Subsequently, the carbon dots must be conjugated to the siRNAs produced. Thus, the siRNAs and carbon dots should be added separately to MES buffer solutions of pH 5.7 with 20 mM glycerol, and then mixed by vortexing to obtain a final solution with a concentration of siRNAs no higher than 12 ng μ L⁻¹ and in a mass ratio with respect to the carbon dots of 40:50 (carbon dots:siRNAs). The solution obtained should be left to stand for at least one hour and then sprayed according to the method proposed above.

V. Spraying

On the other hand, following the same procedure proposed by Schwartz et al. (2020), a low-pressure spray system will be used to release carbon dots conjugated with the siRNAs on plant leaves, allowing high permeation rates into the cells and preserving the integrity of the siRNAs. The spray system employed consists of an Iwata HP M1 airbrush sprayer set at 12 lbs per square inch with fluid setting at 1.5, in addition, BREAK-THRU S279 surfactant is used at a concentration of 0.4 % (v/v), performing 3 sprays in total, on day 1, week 1 and week 2 following formulation preparation. They can be implemented and contrasted with other spraying techniques as proposed by Delgado-Martín et al. (2022), proposing the use of conventional art sprayers adjusted to 2.5 bar pressure for the release of carbon dots conjugated to nucleic acids.

2.3.2.2. Test



Created in BioRender.com

Figure 18. Test protocols' graphic summary

I. siRNAs Electrophoresis

To evaluate the production of siRNAs of 21 nt, fractions are collected every 30 s to perform a run by polyacrylamide gel electrophoresis with TBE running buffer and staining with SYBR Gold, verifying the presence of 21 nt fragments when comparing with the molecular weight marker. After obtaining the 21 nt segments, it is essential to determine the exact retention time at which elution of 21 nt siRNAs begins. In this way it is possible to optimize the recovery process, avoiding the need to re-evaluate the fractions by electrophoresis once this exact time has been determined.

II. RT-qPCR

For the analysis of transcript levels, quantitative PCR with reverse transcription (RT-qPCR) is performed, for which total RNA is extracted from leaf tissue of *T. alata* 5 days after treatment, by extraction with Trizol, and then its concentration is measured by spectrophotometry (NanoDrop). Samples are diluted to 5 ng μ L-1, from which complementary single-stranded DNA is obtained using reverse transcription kits. The products obtained will be used as template for qPCR using primers specific for the 4 CDS of the *T. alata* target genes, in addition to MasterMIX with Taqman probes. A 40-cycle process is performed and the results are quantified by comparing with a reference gene using the comparative threshold (Ct) method: $2^{-(\text{Ct Target Gene} - \text{Ct Reference Gene})}$. Finally, a T-student hypothesis test is performed to compare transcript levels with a negative control that was not sprayed with siRNAs.

III. Northern Blot

To validate the results obtained by RT-qPCR, Northern blotting, a non-quantitative technique for the detection of expression (presence of mRNAs) of target genes in *T. alata*, will be used. Initially, total RNA will be extracted by Trizol, and then those RNAs with poly A segments (representing mature mRNAs) will be isolated by chromatography with Oligo-dT cellulose. Subsequently, the mRNAs are run in agarose gel electrophoresis with formaldehyde, and the bands obtained are transferred to nylon membranes by crosslinking with UV radiation. Finally, hybridization to artificially synthesized radioactive probes is performed for the detection of the target mRNAs.

IV. Size - exclusion chromatography equipped with spectrophotometry.

As mentioned in Built, for the purification of carbon dots it is necessary to perform a size-exclusion chromatography technique, which in turn is also a way to corroborate the formation of these particles when coupled to spectrophotometry. FPLC chromatography is performed on resin filled columns for

filtration and elution of the mobile phase in a solution composed of 50 mM NaCl, monitoring the output solution with spectrophotometric coupling at a wavelength of 360 nm.

2.4. Human Practices

I. Local Problem Statement

The rampant expansion of invasive exotic species is a worrying threat to biodiversity and the stability of ecosystems around the world due to the alteration they cause in the processes that occur within them, in terms of their composition and structure. In particular, the spread of *Thunbergia alata*, known as “black-eyed susan vine” in the Latin American region, and specifically in eastern Antioquia, Colombia, has raised growing concern due to the adverse effects it has on native flora and local ecosystems.

This species, a climber of the Acanthaceae family, native to East Africa and introduced to tropical and subtropical regions of Asia, America and Australia, has adapted to the climatic and ecological conditions of the region, achieving a successful and alarming invasion that appears as beautiful mats on walls, fences and trees, and results in severely affected forests and crops.

T. alata, originally valued in its native habitat for its culinary and medicinal properties, has become a problem species in Latin America due to its ability to colonize large areas and throw native ecosystems out of balance. The introduction of *T. alata* into the Latin American region is the result of its attractive ornamental appearance, characterized by striking orange flowers with ebony-black centers, and its climbing ability that allows it to cling to and grow on supports such as trees and shrubs.

As this species colonizes new areas, it competes fiercely with local species for resources such as sunlight, water and nutrients, exerting pressure on native plant communities and affecting the photosynthetic and CO₂ capture processes of the region's species.

In addition, the plant has a climbing ability that allows it to cover natural supports such as trees and shrubs, limiting its growth and hindering its successful reproduction. Aggravating its high impact, the control of the species is very complicated due to its ability to self-pollinate and the possession of explosive fruits that spray seeds on land where they germinate easily, adding to its reproduction by means of stem and root cuttings that give way to intricate and deep networks that colonize the soil with a dense rhizome, capable of climbing and entangling branches and trunks on itself, other plants and finally extensive areas of forest and planting. At the moment, the only option is to uproot it, but even then, its residues can easily become the stems of new individuals.

The problem becomes a bit alarming when observing its spread at the international level. Its presence in several Latin American countries has generated a challenging scenario for biodiversity conservation. The highly fertile seeds of *T. alata*, capable of resisting and thriving in different climates, coupled with the lack of natural biological controls, has driven its unrestricted expansion. Also, the absence of harsh winters in the Latin American region, with prevalence in countries near the Equator, has allowed this plant to thrive successfully. In places where winters are mild and sunlight is abundant, the seeds of this invasive plant disperse rapidly, generating a rapid and uncontrolled spread, and the lack of limiting climatic factors has allowed it to colonize large territories, suffocating the native flora and disrupting ecosystem functioning with up to 20,000 seeds per cubic meter.

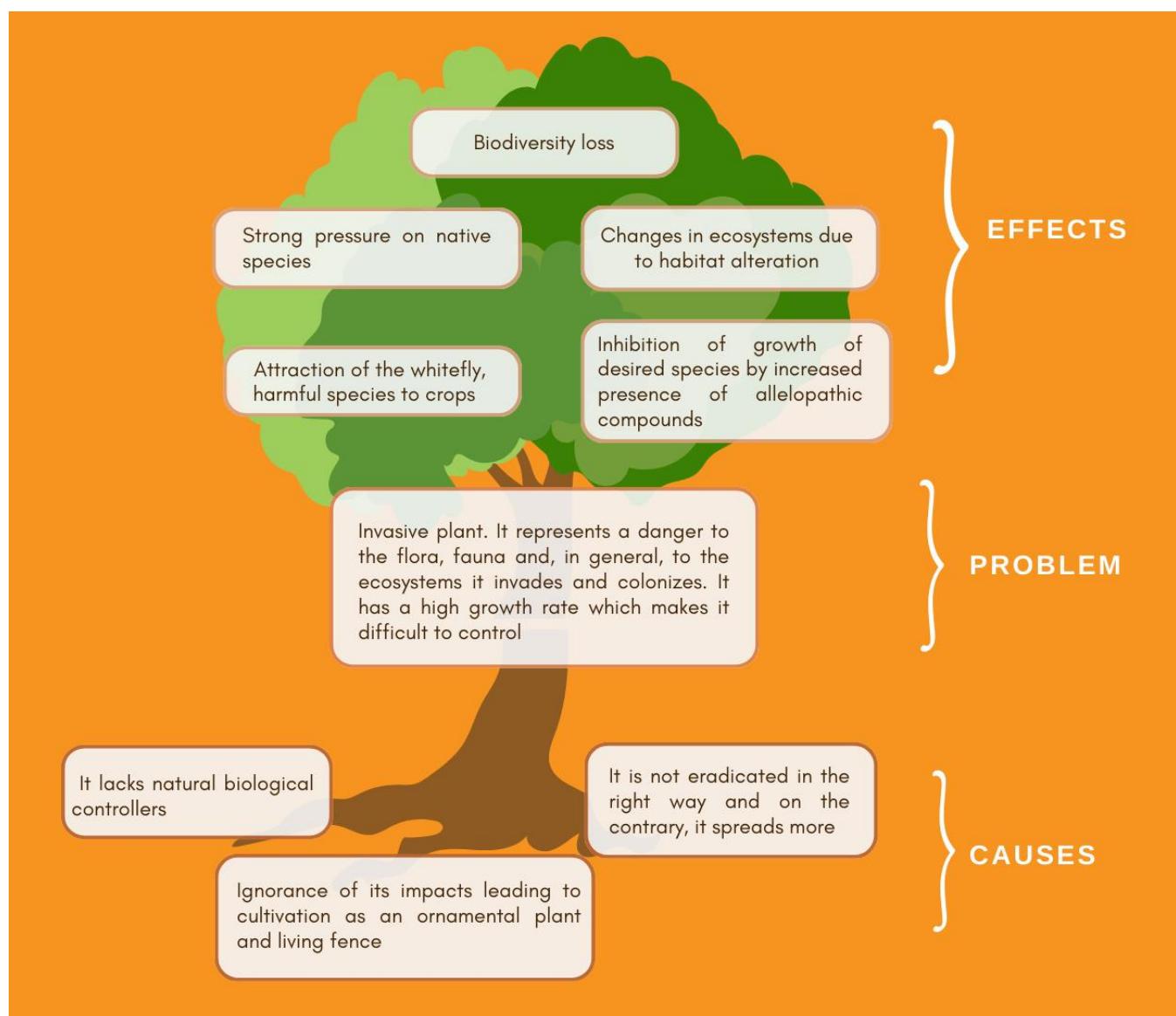


Figure 19. Problem tree

To illustrate the magnitude of the impact of this plant, Brazilian ecosystems such as grasslands and scrublands have shown high colonization in areas with mycorrhizal presence of between 60 and 70%, an association generally observed in the growth of this plant, which confers additional advantages such as drought tolerance, indirect control of root diseases and more efficient and deeper nutrient uptake from the soil. In Ecuador, more than half of the territory has the ecological parameters for the development of *T. alata*, and it has been reported how the vegetation and river systems have been covered with an "orange blanket", with 1,375 observations reported up to April 2023 and unregulated commercialization.

Now, this invasive species in the Colombian context has been recognized by the Instituto de Investigación de Recursos Biológicos "Alexander von Humboldt" as one of the ten most problematic species and is registered throughout the national territory, between 1500 and 2900 meters above sea level. Locally, in the highlands of eastern Antioquia, with observations from 1500 to 2900 meters above sea level. This invasion has generated additional concern, since the microbiota of the region's soils, together with a high mycorrhizal colonization and other characteristics described above, are allies of its growth and dispersal. It also has generated additional concern, since the microbiota of the region's soils, together with a high mycorrhizal colonization and other characteristics described above, are allies of its growth and dispersal.

Given this scenario, a critical question arises: how to effectively and sustainably address the environmental problems caused by the invasion of black-eyed susan vine in eastern Antioquia, Colombia? The present research project is proposed as an answer to this challenge, with the purpose of designing a bioherbicide through synthetic biology.

II. Environmental education

The lack of knowledge about the serious consequences of *T. alata* in the Colombian Andean Forest means that the problem is not visible on a large scale. Besides the ornamental uses that have been given to this plant, so that most people have a positive concept about it, its beautiful and exotic colours make *T. alata* welcome and desired to decorate spaces, causing the expansion of the same through the territory. For this reason, throughout the development of the project, it was identified that the lack of environmental education in the population is a primary factor for the colonization of the plant to increase every day and bring with it the deterioration and damage to the native forest species.

Therefore, it was necessary to develop different activities and dynamics to inform the community about the problem, and in conjunction with the communication component of science, were designed to showcase synthetic biology and its multiple qualities in providing positive biocontrol, and also to get people to take over the care and control of invasive species in the country; when a problem is understood

and recognised, a sense of belonging in the community develops, which will spread knowledge and thus create awareness, and impact the environment in a positive way.

It is important to understand that this problem is the result of ignorance and lack of legislation regarding the proper management and use of exotic species, so it is essential that those who are unaware of the effects of *T. alata*, be informed and educated, and thus work together for the eradication of the black-eyed susan vine in eastern Antioquia.

The activities carried out in the human practices section were important to help the members of the group to realize that the relationship with the community, companies and experts are very important to continue with the whole process of each of the areas of competence, both in science communication, arts and even in research. Knowledge is not only imparted from the SynthEIA group to the community but also the other way around, since it was a mutual learning process where the group also obtained new information.

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Environmental education is a key factor in preventing the problem from worsening, so activities were carried out in towns in eastern Antioquia, such as Jardín and Marinilla, to transmit the knowledge gained during the *T. alata* project to the community through the distribution of leaflets and discussions with the community.

III. Implemented activities

The project not only focused on developing a proposed solution based on synthetic biology, but also had as a fundamental pillar the awareness of the community about this invasive species and its negative impacts on the local ecosystem. For this reason, human practices were used that brought together various factors related to ethics, society, and environment.

Awareness campaigns and educational workshops were organised in schools and universities to promote understanding, awareness and active participation. Extra-curricular activities were also carried out to bring the issue closer to the community, such as a photography competition to raise awareness of the

abundance of this species in the region, and games to help the community understand the purpose of Poeticide.

Environmental education is a key factor in preventing the problem from worsening, so activities were carried out in towns in eastern Antioquia such as Jardín and Marinilla in order to transmit to the community the knowledge acquired during the project on *T. alata* through the delivery of flyers and conversations with the community.

In the campaign for this education in the park of Jardín, information was shared through a presentation, and it was a space in which the public was invited to reflect on the invasion and eradication of *T. alata* in the sector and in Marinilla, there was an approach to the community in the market square, flyers were distributed, and people were talked to know if they know how dangerous it is and teach them manual techniques that contribute to contain it.

Also, consideration began to be given to the various possible effects of the proposed solution through synthetic biology on the natural environment by considering comprehensive environmental risk assessments to identify possible ethical implications, the implementation of such a solution. There was also a commitment to seek out experts in ethics and environmental biology, including consulting with expert committees on the subject, environmental entities and universities, to ensure that each action was ethically sound and socially responsible.

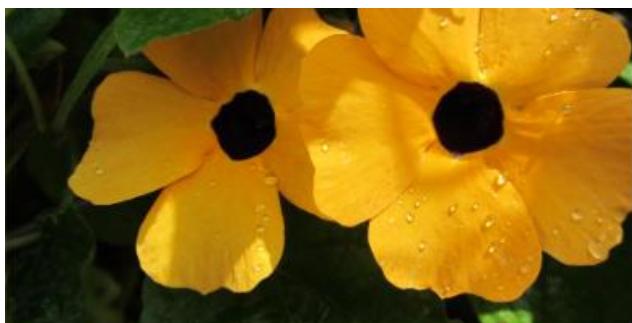
2.5. Integrated Human Practices

I. Activities to identify the problem

The following activities were developed to identify the international and local perception of the problem:

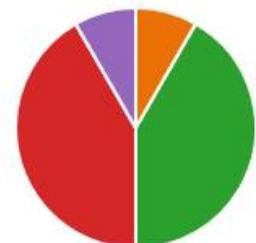
- International survey**

Although the problem extends throughout Latin America, not everyone knows about this problem and its detrimental effect on the ecosystem, which is why the decision was made to develop a survey that was shared with the iGEM design league community. A response was obtained from teams from Mexico, Brazil and Peru where the assumption was confirmed, 100% of the respondents answered that they do not know or do not consider this plant a risk to biodiversity and 97% consider this plant beautiful or ornamental (Figure 20). This led to the conclusion that there is indeed no generalized environmental awareness, it is not known to the general public that work is being done in other places to control this plant and that is the reason for so little information about it.



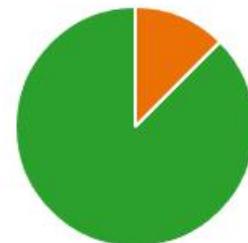
What do you think of this plant?

Good for the environment	0
Invasive	1
Ornamental plant	5
Gorgeous	5
Another one	1



Do you think that this plant is considered a risk for the biodiversity in your country?

Yes	0
No	1
No idea	7



SynthEIA: Poeticide

Hi! We are an iGEM team from Medellín, Colombia. We would be very grateful if you help us answering some questions.

Figure 20. Poeticide international survey results conducted in Office Forms

- **Field trips**

Field trips were carried out to document and identify the distribution of Black-eyed Susan Vine in order to generate a direct approach to the problem by talking to local people. Visits were made to several towns in Antioquia, including Santa Elena, Guarne and Santuario, which exhibited a high density of invasive populations, as shown in Figures 2 and 3. During the visit to Santuario, a conversation was held with representatives of the environmental authority Corporación Autónoma Regional de las Cuencas de los Ríos Negro y Nare (CORNARE), who presented their perspective on the problem.

In addition, the presence of the invasive plant was identified in various departments of the country, as shown in Figure 3. In particular, its presence in Cundinamarca confirms what experts have pointed out about the ease of adaptation of this plant in the Andean zone. This situation is of serious concern because it leads to an increase in ecological problems, including the displacement of native flora and the consequent loss of biodiversity in the region's forests.



Figure 21. Photos of Guarne and Santa Elena, Antioquia



Figure 22. Photos of Suesca, Cundinamarca and Guarne, Antioquia

- **Visits to companies and foundations**

Medellin and the eastern Antioquia region are known for their significant flower production, which prompted visits to flower companies. During this phase, visits were made to two specific companies: Flores El Trigal SAS, specialized in the production of flowers for export, and ICON Selections (Figure 4), focused on the improvement of Chrysanthemum through hybridization processes. These interactions provided the opportunity to share ideas and receive valuable feedback from them.

In addition, a visit was made to the Botanical Garden of Medellin, an institution committed to biodiversity conservation, and which has carried out several initiatives to raise awareness about the problem of invasive species, including talks with experts. During the visit, several members of the team participated in the conference entitled “Historia, vida y poderes de una especie invasora: el caso del ojo de poeta” (in English, “History, life and potential of an invasive species: the case of the Black-eyed Susan vine”), led by Mario Quijano, leader of the research group in floristic studies and herbarium of the Universidad Católica de Oriente. This experience provided the team with the opportunity to establish meaningful connections that later led to a meeting with the research group, in which we gained knowledge on the subject and gained a deeper understanding of why this invasive plant has been so successful.



Figure 23. Visit to ICON Selections

II. PESTEL analysis

As shown in Figure 24, the context in which the country finds itself is indeed diverse. On the one hand, there is a government that implements policies that could benefit the company economically, but that comes from an economic recession and can generate a certain economic imbalance. In the social environment, the company could be well-received by a certain young population that follows trends and is concerned about the well-being of the environment, while the social gap would prevent it from reaching certain rural populations due to economic and educational factors. In another aspect, it can be evidenced that in Colombia research and technology are existing fields of action, but there is still a lot to be explored and even more when it comes to private companies interested in this topic, this is why there would be an opportunity to contribute in this aspect. The legal requirements for the application of our product are not clear since it is an innovative product that aims at the conservation of native biodiversity.

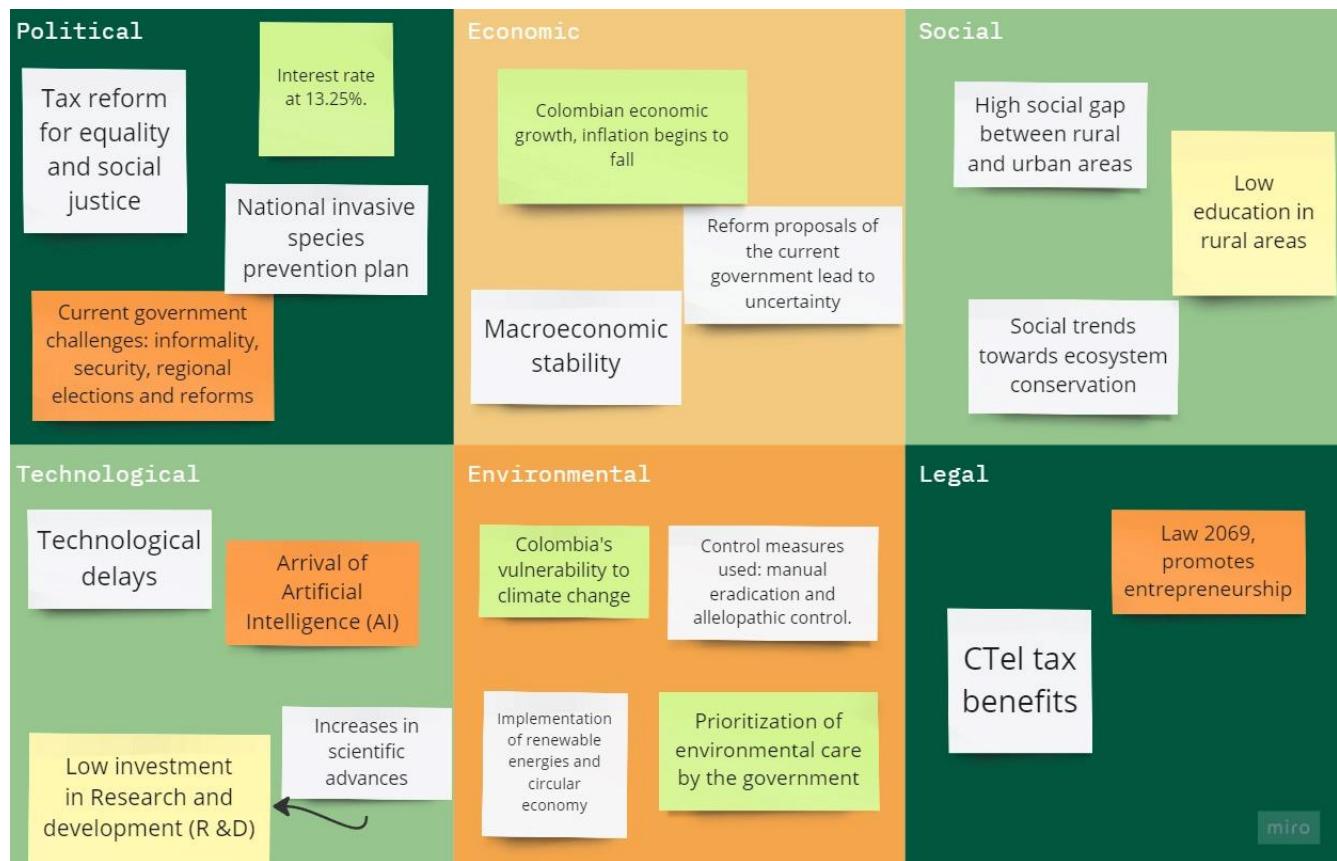


Figure 24. PESTEL analysis

III. Stakeholders identification and analysis

SynthEIA as a research group aims to ensure that its potential stakeholders are well-informed about the Poeticide project. Therefore, it was crucial to identify these potential stakeholders and assess their

significance and potential contributions to the project. This was done with the intention of developing effective engagement strategies that can collect valuable feedback to enhance the proposed solution.

As shown in Figure 25, stakeholders were classified as internal and external; the external stakeholders that were prioritized consisted of government entities, the local community and experts on the problem.

Thus, communication was agreed with the Floristic Studies research group of the Universidad Católica del Oriente, locally recognized for dealing with the black-eyed Susan vine problem based on the morphological, reproductive and distributive analysis of the plant in Eastern Antioquia and other parts of the country, in order to arrange a meeting to exchange knowledge on the problem of the invasion of *T. alata*. During the meeting, we presented the proposed solution using synthetic biology and with the help of the research group we were able to detect key factors to take into consideration; in the [attached link](#) you can watch a video with the questions we consider most relevant for the experts in order for us to better understand the problem.

In addition, a meeting was agreed and conducted with CORNARE, the environmental authority that is in charge and has the authority to deal with the problem of invasive species in the territory of Antioquia; in this space, the team was able to present the project to the people in charge of the office of biodiversity management, protected areas and ecosystem services; starting with the problem that is widely recognized by this institution, and showing the proposed solution.

In response to this, the individuals responsible for the discussion shared their insights regarding the need to address the issue of specificity in order to prevent adverse effects on native fauna and flora. They also emphasized the significance of collaborative initiatives aimed at achieving a common objective.

Environmental awareness through art acquires a fundamental importance; people who were working with the invasive plant were identified from the production of a short film with the main theme of black-eyed Susan vine, and interviews were conducted to understand why they chose this theme, as well as asking other relevant questions as can be seen in the [following link](#).

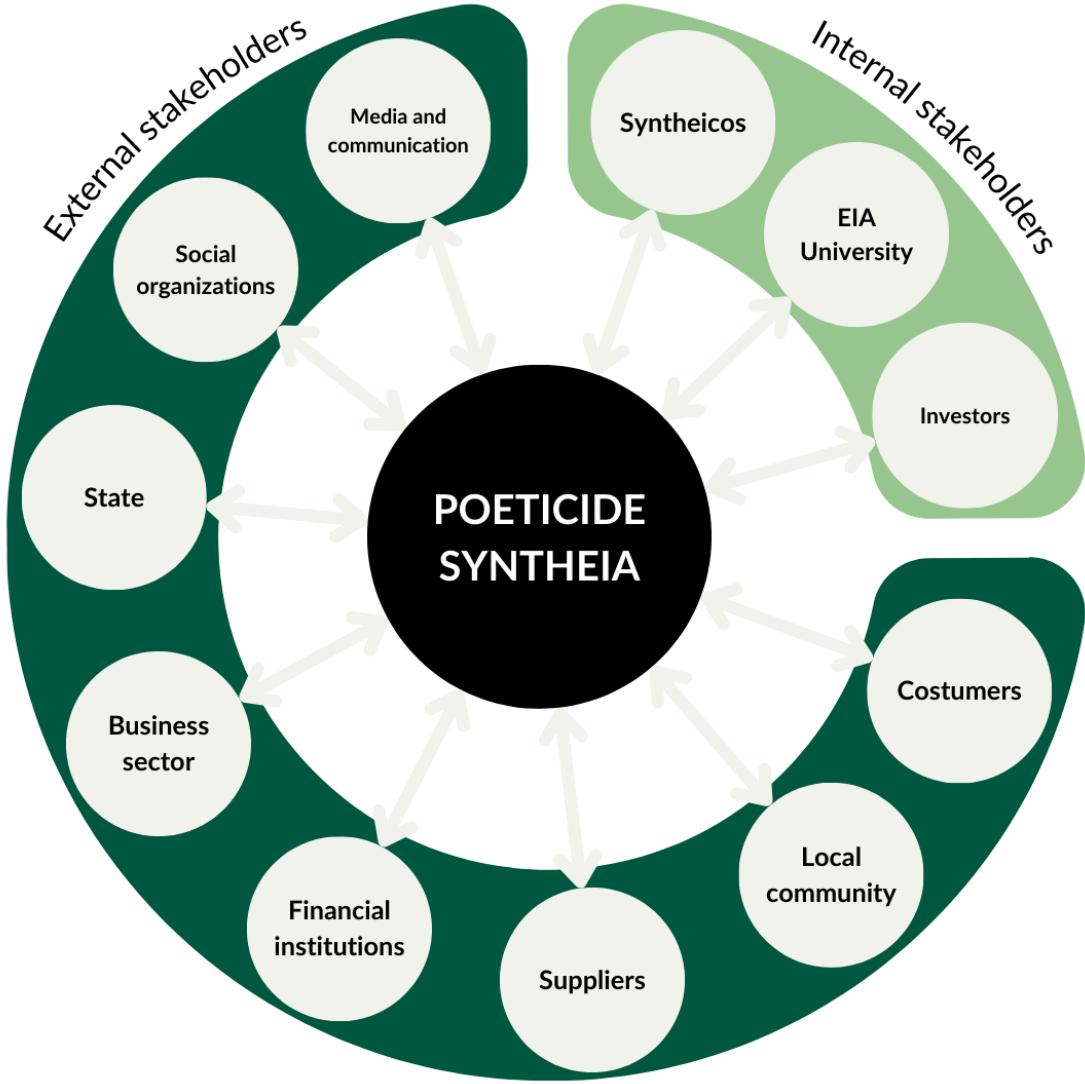


Figure 25. Stakeholders map

IV. Diversity and inclusion

In the case of internal stakeholders SynthEIA, a multidisciplinary team of EIA University students, mostly made up of women (approximately 75%), focuses on gender equality in STEM, i.e. equal rights, responsibilities and opportunities in the disciplines of science, technology, engineering and mathematics. Globally, in 2019, only 29.3% of female researchers and 35% of STEM students were women, but in Latin America and the Caribbean, the number of female researchers rises to 45%, although they remain a minority. Gender inequality in STEM is a concern, as it is estimated that 50% of future jobs will be in this field, and inequality skews research.

Since the majority of SynthEIA members are women and there is a significant gap in female representation in STEM, the team is contributing to the diversity of the team. According to UNESCO

data (2020), only 27% of countries worldwide have reached "gender parity", equal proportional representation of men and women, in research, where women represent between 44% and 55% of researchers. Colombia is close to reaching parity, with 37.3% female representation.

What was the project like before and after integrating knowledge of human practices?

Before integrating human practices insights and collaborations into the project, the proposed solution did not consider the specificity needed for the proposal to be viable. This situation happened because the microorganism selected as a chassis, was going to be released into the environment and it was considered to be specific for infecting the plant *T.alata*, but after agreeing to talk to some people who were involved with this problematic (for example the professor Mario Quijano), their comments made the team realize two things; the first one was that with the little knowledge that appeared about this microorganism, it was impossible to control the fact that it could infect other plants and secondly it was also hard to find more molecular and genetical information on the plant, so if synthetic biology was going to be used to alter the microorganism to eradicate this plant it was also difficult to predict if the metabolite in charge of eradicating the plant was very specific.

From this point on, the investigation team started searching for different solutions and the project changed into something more specific and that was a little bit more well seen by the stakeholders of the project, because the problem of specificity was sorted out and they were able to recognize it. The changes made to the project, or the idea for the product involves two things, ARNi and Carbon dots, which means that a microorganism is not going to be introduced in the environment and it will only be used as a media for production, so technically it is safer for the ecosystem and easier to control.

2.6. Solving Local Problems

I. Existing solutions: Strategies implemented for control and industry contributions.

Existing solutions to address the problem of the invasive species *T. alata* are based on a series of strategies and contributions from industry. Previous research has highlighted the exceptional growth habit of this species, which allows it to climb various supports and colonize them effectively, covering them with dense layers of stems and leaves (Quijano, 2019). In Colombia, ecological, social, cultural factors and the richness of nutrients in soils have facilitated the invasion of this plant, which has led to the formation of dense "mats" in places such as eastern Antioquia, covering areas of land and banks of water sources.

It is important to keep in mind that, although the negative impact of invasive species on biodiversity is widely recognized globally, in Colombia there is still considerable misinformation and lack of knowledge on this subject. Therefore, a crucial first step for the control of this plant must be to strengthen biological research and education on these species. The Alexander Von Humboldt Biological Resources Research Institute, for example, has contributed to increasing the visibility of this problem by including Black-eye susana vine as one of the 35 species of exotic flora that represent a threat to the country's endemic biodiversity in a report published in 2010.

Además de los esfuerzos de investigación, en el país se han implementado estrategias a través del Ministerio de Ambiente y Desarrollo Sostenible, que en 2011 creó el Plan Nacional para la In addition to research efforts, strategies have been implemented in the country through the Ministry of Environment and Sustainable Development, which in 2011 created the National Plan for the Prevention, Control and Management of Introduced, Transplanted and Invasive Species. The objective of this plan is to promote biodiversity conservation through the development of policies, the creation of programs for the prevention and eradication of invasive species, the encouragement of research and the monitoring of these species, as well as raising awareness. These actions represent a comprehensive approach to address the problem and seek effective solutions in the fight against invasive species, such as the black-eye susane vine.

In addition to research efforts, strategies have been implemented in the country through the Ministry of Environment and Sustainable Development, which in 2011 created the National Plan for the Prevention, Control and Management of Introduced, Transplanted and Invasive Species. The objective of this plan is to promote biodiversity conservation through the development of policies, the creation of programs for the prevention and eradication of invasive species, the encouragement of research and the monitoring of these species, as well as raising awareness of these species. These actions represent a comprehensive approach to address the problem and seek effective solutions in the fight against invasive species, such as the black-eyed susan vine.

On the other hand, from public institutions such as Cornare, Coranquioquia and Corpoboyaca, several efforts have been made to make this invasive species visible, strategies have been given for its eradication which consist of:

- 1. Basic measures for their management:** It is recommended to refrain from planting these species and fundamentally to avoid transporting its soil, known as black soil, from one place to another, since

this acts as a vehicle for the propagation of seeds and rhizomes, which contributes to the continuous expansion of the species (Cornare, 2011).

2. **Basic control measures:** To effectively address the control of these invasive species, manual eradication is recommended, extracting both the plants and their roots, and depositing the residues in a compost bin. This facilitates the elimination of seedlings that can germinate, while at the same time becoming compost. It is essential to pull up the bushes in their initial state and avoid accumulating pruning residues near streams and rivers, which are favorable areas for their reproduction. Likewise, avoid dumping waste from grassland management in the forests, as this contributes to the invasion of these species. Avoid the use of soil (black soil) from open areas and pastures, as well as grass, as this can contribute to their dispersion and invasion in natural forest ecosystems. On the other hand, it can be used as fodder for confined animals in a tender state, which can be a sustainable option for its control. Finally, if there are orchards where the plant is present, it is recommended to plant other plants such as Artemisia or Yerbabuena, because these exert an allelopathic control (Cornare, 2011).

II. Why to use synthetic biology for *T. alata* biocontrol?

Synthetic biology is an emerging field that combines principles of biology, engineering and technology to design and build novel biological systems or modify existing biological systems with the goal of achieving specific functions. In the context of invasive plant eradication, synthetic biology could offer several advantages and innovative approaches (Koukara & Papadopoulou, 2023):

1. **Specificity:** Synthetic biology allows the design of biological systems that are highly specific to the invasive plant in question. This means that organisms or agents could be created that only affect the invasive plant and do not harm native species or the natural environment.
2. **Precision:** Through controlled genetic modification, an organism could be designed to attack specific characteristics of the invasive plant, such as its reproductive mechanisms, growth or herbicide resistance, which would increase the effectiveness of control.
3. **Reduction of collateral damage:** Conventional methods of invasive plant control, such as the use of chemical herbicides, can have adverse effects on the environment, affecting native species and causing ecological damage. Synthetic biology could minimize these collateral damages by being more specific in its action.
4. **Sustainability:** By designing biological systems that are self-limiting or deactivated after their function, synthetic biology could reduce the persistence of the modified organisms in the ecosystem after eradication of the invasive plant, which would promote long-term sustainability.

5. Adaptation to changing challenges: Synthetic biology could allow for real-time adjustments as the invasive plant evolves or adapts to traditional control methods, providing a more flexible strategy for maintaining control.

III. Strategies and methods for measuring the impact of our project on our community

Before conducting any field tests, it is necessary to have laboratory tests to ensure the effectiveness of the bioherbicide and that it does not represent a risk to the native fauna and flora or to the local community. Then, to evaluate the impact of the project on the community and the ecosystem, a series of comprehensive strategies and methods have been designed. First, the location and spread of the invasive plant will be monitored at different stages of the project, using geographic information systems (GIS) and mapping applications to document its presence and the effectiveness of control measures, which could be done in conjunction with research groups such as the Floristic Studies research group of the Universidad Católica del Oriente.

At the same time, biodiversity studies will be conducted before and after project implementation to understand how the eradication of the invasive plant impacts other plant species, animals, and microorganisms in the area. In addition, soil and water quality in the areas affected by the invasive plant will be analyzed to detect possible changes as a result of eradication. Community opinions and observations will be collected through surveys and questionnaires before and after the project, and the economic costs associated with eradication will be evaluated against the benefits derived from land and resource recovery. To ensure that eradication is sustainable, the area will continue to be monitored for several years after implementation.

Collaboration with the local experts mentioned above, as well as botanists and ecologists, will provide an accurate assessment of the impact on the ecosystem. Specific key performance indicators (KPIs), such as reduction of invasive plant cover, recovery of native species and improvement of soil quality, will be defined and these indicators will be constantly tracked throughout the project. These strategies and methods will provide a full understanding of the project's impact on the community and the environment.

2.7. Impact on the Sustainable Development Goals (SDGs)



Figure 26. Impacted SDGs

The United Nations adopted the 17 Sustainable Development Goals in 2015 to stop poverty, safeguard the environment, and ensure universal peace and prosperity by 2030. As a result of it, it is possible to say that this project has the potential to influence three of these SDGs: the second goal (zero hunger), the thirteenth goal (climate action), and particularly, the fifteenth goal (life on land).

Even though the 2nd goal refers to ending hunger, achieving food security and improving nutrition and our project does not directly relate to food, it is possible that the invasive species the project is treating could endanger some food resources. Globally there haven't been reported cases in which this plant affects power crops, but locally it is pretty visible that the plants are growing very near the crops used as food source, having a slight possibility of damaging them by reducing the available nutrients, so with the solution, this risk can be eradicated. The 13th goal, on the other hand, urges people to take action to combat climate changes and its impacts. Our project contributes to the fulfillment of this objective by protecting native forests from invasive species that can greatly affect ecological dynamics.

Finally, the most important goal "A fundamental shift in humanity's relationship with nature is essential", in which our project aligns, it is the 15th one, that refers to the protection, restoration and the promotion of the sustainable use of terrestrial ecosystems, a sustainably manage of forests, to reverse land degradation and stopping biodiversity loss. Because the problem we are trying to solve with the synthetic biology solution, refers to an invasive plant that is endangering the native flora of the places where it has landed on.

2.8. Responsibility: Safety and Security

Genetic manipulation and editing of *Thunbergia alata* is usually carried out in a biosecurity level 1 (BSL-1) laboratory. This is because *Thunbergia alata* is a non-pathogenic plant and does not pose a significant risk to operators. However, it is essential to have good laboratory practices and take appropriate precautions to avoid genetic contamination. To perform these procedures, standard laboratory equipment and utensils such as microscopes, micropipettes, incubators, and autoclaves shall be used for instrument sterilization. In addition, specific gene editing procedures will require additional tools, such as reagent kits and plant tissue culture techniques, to achieve the desired genetic modification in *Thunbergia alata*.

This project involves three fundamental parts: carbon dots as carriers, *Escherichia coli* as the chassis and RNA as the product of the genetic modification, so it is fundamental to follow safe protocols and practices to ensure ecosystem integrity and the safety of the personnel involved. The procedures and the reasons why there is no serious risk in this type of project are described as follows:

1. Carbon Dots Management: Carbon dots are carbon nanostructures that are used in a wide variety of applications, such as fluorescent markers. For safe handling, standard safety guidelines for nanomaterials must be followed, including working in a suitable laboratory environment, using personal protective equipment, and carefully monitoring waste disposal.
2. *Escherichia coli*: this type of bacteria, although it may include pathogenic strains, is commonly used in laboratories and is a fundamental tool in biotechnology. To avoid risks, rigorous handling practices must be followed, such as proper sterilization of equipment and use of laminar flow hoods to minimize aerosol dispersion.
3. RNA (Ribonucleic Acid): RNA is an essential molecule in molecular biology and genetics. Safe handling involves working under controlled conditions to prevent sample degradation, using sterile gloves and equipment to prevent cross-contamination.

Some reasons why the project would not have a negative or hazardous impact on the environment are as follows:

- Control of the environment: Scientific research laboratories are designed to control and minimize risks. Procedures are carried out in controlled environments, reducing exposure to potentially hazardous organisms or substances.
- Safe practices: researchers follow rigorous safety protocols, including training in the safe handling of substances and microorganisms, thus reducing the risk of accidents.

- Proper waste disposal: Waste generated in these projects must be disposed of in a safe manner, following the corresponding environmental and biosafety regulations.

Safety in these types of research projects is based on the application of safe practices and risk control in specialized laboratory environments. When proper guidelines are followed, risks are minimized, making these projects safe for both personnel and the environment.

2.8.1. Safety Form

1. Describe the goal of your project: what is your engineered organism (or other synthetic biology product, system, or tool) supposed to do? Please include specific technical details and names of important parts.

In order to successfully eradicate the invasive plant *Thunbergia alata* from the invaded areas, our study aims to alter and exploit the fungus *Alternaria thunbergiae*, giving it the ability to recognize the presence of a metabolite of the plant, overproduce the protein Nep1 and induce necrosis.

However, for lack of information about this fungus, the idea migrated to the biocontrol the invasive species by using recombinant interference RNAs produced in *Escherichia coli* by designing a genetic circuit that facilitates high levels of siRNAs production, which, when conjugated with carbon dots and released by spraying, will permeate *T. alata* cells, and effectively silence certain genes relevant to the lipid metabolism of the invasive species.

2. Would any of your project ideas raise safety concerns in terms of: Harm to agricultural animals, crops, or domesticated animals, Harm to the environment, including wild plants and animals, Harm to the researchers working with this technology or the public?

None of the above.

Our project aims to selectively induce harm in a wild and invasive plant, *Thunbergia alata*, through the use and genetic modification of a pathogenic microorganism owned by the plant, *Alternaria thunbergiae*, which does not report pathogenicity in other species, including humans. For further prevention of any side damage, the transformation will be induced by a metabolite whose production is only by the plant in question and another species of *Alternaria*, which is not native to the region either.

However, the idea of the project changed, now through iRNA it is planned to inhibit the expression of specific genes of *Thunbergia alata*, by binding and blocking the translation of complementary mRNA. This gene silencing, being specific to the black-eyed susan vine, does not generate a potential risk for the

other plants living around it. Moreover, since iRNA is such a weak and weak molecule, the risk that it could affect life around it is almost zero, as this molecule degrades very easily with environmental conditions.

3. Do any of the new parts or devices that you designed this year raise safety concerns? If YES:

a. Could any of your parts be hazardous on their own and/or in the context of your project?

Parts with no hazardous function in their organism of origin, but that might be hazardous when used in your project (e.g. quorum-sensing circuit that triggers release of an insecticide).

For our new solution, the answer changed: None of our parts could be hazardous.

b. How do you plan to handle the safety concerns once you are in the lab?

In order to achieve this, and in light of the fact that *Alternaria thumbergiae* (chassis) and Agrobacterium tumefaciens (transformation mediator) are not harmful to human health, we will adhere to the standards and guidelines of the biosafety manual developed by the WHO, working in a level I biosecurity laboratory (low or zero individual and population risk), with a class I laminar flow chamber, entry and exit registration of pre-authorized personnel, good as: • Gloves • Safety glasses • Closed shoes • Long trousers • Laboratory cap If the use of a microorganism similar to the chosen chassis, *Alternaria thumbergiae*, such as *A. alternata*, which has moderate pathogenicity in humans, is required, a safety laboratory level II would be used.

Even if the solution proposal changed, we are planning to handle the same safety concerns once we are in the lab.

c. How, if at all, have safety concerns influenced your experimental design and are there any other actions (such as stakeholder consultations or developing a responsible communications plan) that you plan to take to manage safety concerns?

Given that we will work with a pathogenic fungus, we are aware of the environmental risks that this escape from work and experimentation environments may involve, so we are constantly consulting and enforcing the relevant local and international regulations for our project, such as the Cartagena Protocol. In addition, support, training and supervision will be requested from the local environmental authorities that govern the area of impact of the invasive plant, Corantioquia and Cornare, informing them about the work and implementing responsible communication plans with the relevant entities.

For the new solution we will consider the same safety concerns.

d. How could other teams learn from your experience? (optional).

As a team we have had the lucky experience of taking courses where the main objective was to learn how to develop biosafety protocols for levels 1 and 2 laboratories, achieving that our university adopted them as a general standard for all students when entering these spaces. With this in mind, other teams could come to us to guide them in the development of their own protocols. Furthermore, the problem that is being addressed involves having an important knowledge of the interactions between several species in the same ecosystem, which is very important from an environmental point of view when making any intervention in these. Additionally, the interaction with regulators and the regulation needed to carry out these interventions is quite important knowledge that, although it may vary from country to country, it is essential to socialize them so that researchers always take these regulations into account.

4. Is there a local biosafety group, committee, or review board at your institution?

a. Have you consulted your local institutional biosafety group? What do they think about your project?

Our mentors are the leading teachers and responsible for the biosafety and the monitoring of the laboratories in our university, so they are always informed and in constant approval of biosecurity of what is raised throughout the project. In addition, we have a team of trained laboratory technicians who constantly support and evaluate us. On the other hand, our university has a bioethics committee that looks after the biological safety of the projects, ensuring that they are framed within the biosafety standards according to the possible impact they may have on humans and the environment.

b. Which biosafety rules or guidelines would you have to consider in your country if you were to carry out your project in the lab? What entity regulates these in your community?

In Colombia, we must be guided by:

- Law 740 of 2002 adopting the Cartagena Protocol on the Safety of Biotechnology of the Convention deals with Biological Diversity.
- Article 18 of Decree 4525 of 2005 establishing the National Technical Committees on Biosafety.
- Law 1926 of 2018 approving the Nagoya – Kuala Lumpur Protocol on Supplementary Liability and Compensation to the Cartagena Protocol for the Safety of Biotechnology.

The entities that regulate biosafety in our country are:

- National Technical Committee on Biosafety for Living Modified Organisms for Environmental Purposes.
- Ministry of Environment and Sustainable Development.
- Ministry of Science, Technology and Innovation.
- Invima .
- Cornare (Departmental) .
- Corantioquia (Departamental).

5. Have any of your team members (students) previously performed a risk assessment?

Yes, and filling out this form helped us learn more about risk assessment.

2.8.2. Additional insights into biosafety and biosecurity

The initial idea of the project, to use the fungus *Alternaria* to perform biological control of *Thunbergia alata*, was completely changed due to different problems that were presented to us: lack of genomic sequencing information on both *Alternaria* and *Thunbergia*, gaps on the specificity of the attack of the fungus on the plant, and great challenges to ensure that the bioherbicide did not cause harm. This is why our project migrated to the idea of gene silencing with iRNA, giving us more clarity on the specificity of attack, and a double assurance given that that the iRNA molecules such as siRNAs degrade rapidly. It is also important to add that, with this new idea, it is not necessary to use level II microorganisms or higher.

We began to develop the idea consulting and applying local and international regulations on biosafety that are relevant to our project, such as the Cartagena Protocol. In addition, we have sought advice from other organizations and environmental authorities in the country (such as Corantioquia) and other universities like the “Universidad Católica de Oriente (UCO)” in order to have a continuous interdisciplinary evaluation of the possible biosafety risks that our project may generate, with the objective of correcting them in time so that they do not transcend as a possibility. We plan to collaborate with government institutions such as CORNARE in order to follow the national and local regulations, and to have access to local communities. We could learn a lot hearing the opinions of the citizens of those places where *T. alata* represents a severe problem and collaborating with those institutions, finding real solutions for society.

We estimate that our project can be of great help to our community and its challenge to eradicate Black-eyed Susan vine. For after the competition we plan to enhance our knowledge in synthetic biology, and we hope that our experience in iGEM Design League will be a start of a more solid research group. We also hope that the project can advance beyond just design, to be able to give relief to native plants and crops and free them from this invasive plant.

In addition, with the collaborations we did (Colombian Network of Synthetic Biology, talks on invasive plants), we were able to leave a more solid foundation for our next project.

3. Multidisciplinary Excellence Section

3.1. Collaboration and Partnership Efforts

During the season, our group forged valuable collaborations with several groups such as: Fenix (participants from previous years), igem UAM, Theobroma igem, igem UDEA teams and igem biowulf. Our primary objective was to engage in knowledge exchange and gain insights from experienced participants in the iGEM competition and synthetic biology. These collaborations had essential insights into competition dynamics, human practices, research methodologies, participation in webinars with fellow groups, talks about our genetic circuits and proposals to share the projects.

Each collaboration yielded distinct outcomes, but the most remarkable ones were the following:

- With the Theobroma team we initiated discussions on the establishment of the "National Network of Synthetic Biology Students" in Colombia, uniting various stakeholders to promote synthetic biology awareness in Colombia. With them we also inaugurated the Virtual Colombian Symposium in Synthetic Biology. It was made with several other groups and we got to know more about their projects.
- We also formed an important partnership with the team igem UAM, because both groups shared a core focus on addressing issues related to invasive plants. While igem UAM explored the potential of the plant *E. crassipes*, we were dedicated to eradicating the plant *T. alata*.

In these meetings, collaborative plans were established to raise awareness about invasive plants in Mexico and Colombia. The efforts included Instagram campaigns, stories, and strategies to engage people in everyday life, culminating in a webinar dedicated to issue the problems with invasive plants.

3.1.1. Collaboration efforts

Throughout the whole season synthEIA collaborated with four groups: Fenix, igem UAM, Theobroma igem, Biostyvia and igem UdeA team, with the objective of doing an exchange of opinions and the goal of learning from people who have been before in the competition or have experience with synthetic biology, because the group is new to the competition. The outcomes of these collaborations were very rich with basic knowledge about the competition, such as handling human practices, the investigation process, model struct and also the webinars made with other groups.

The following are the outcomes that the group got from every interaction.

Fenix collab: Activities for integrating both groups, solving doubts about the competition and propose a strategy for the promotion of synthetic biology in Colombia.

Igem UAM: promised to help out with human practices and biological circuits, collaborations for environmental awareness and highlight native flora.

Theobroma team: mentoring with the part of investigation, talks about the “red nacional colombiana de estudiantes en biología sintética”

Igem UDEA team: collaboration for the divulgation of synthetic biology in Medellin, tips for letting the universities know the projects.

Igem biowulf: We shared the research part of the project in order to advise us on the generic circuit, biobricks and mathematical model. This collaboration provided important knowledge about the bases to take into account for the development of the mathematical model.



Figure 27. SynthEIA collaborations

During the meetings, discussions revolved around organizing a significant gathering of Colombian groups. With the assistance of the Theobroma team leader, it was possible to create the National Network of Synthetic Biology Students; a group with different actors, each with the primary objective of

promoting awareness of synthetic biology within the country. The consensus among these groups was that the topic remained relatively unfamiliar.

One of the main events from this newly created group was the first virtual Colombian symposium in synthetic biology: The mission encompassed a multifaceted approach, to further the promotion of synthetic biology in Colombia, while also fostering the spread of this discipline across the region. The participant groups aimed to strengthen collaborations between various universities, whether or not they were participating in iDL 2023, both nationally and internationally. Sharing research progress achieved within each iDL project and in the broader realm of Synthetic Biology stood as a core objective. Additionally, they aspired to create an optimal platform for each team to present their scientific communication posters to a virtual audience. The groups participating in the event were: iGEM GeneSys (Los Andes - Bogotá), iGEM SynthEIA (EIA - Medellín), iGEM UdeA (UdeA - Medellín), iGEM Biomarkers (Perú), UAM (México), Biotech EC (Ecuador) and the anfitrions iGEM Theobroma (UDES - Bucaramanga).



Figure 28. Creation of the National Network of Synthetic Biology Students

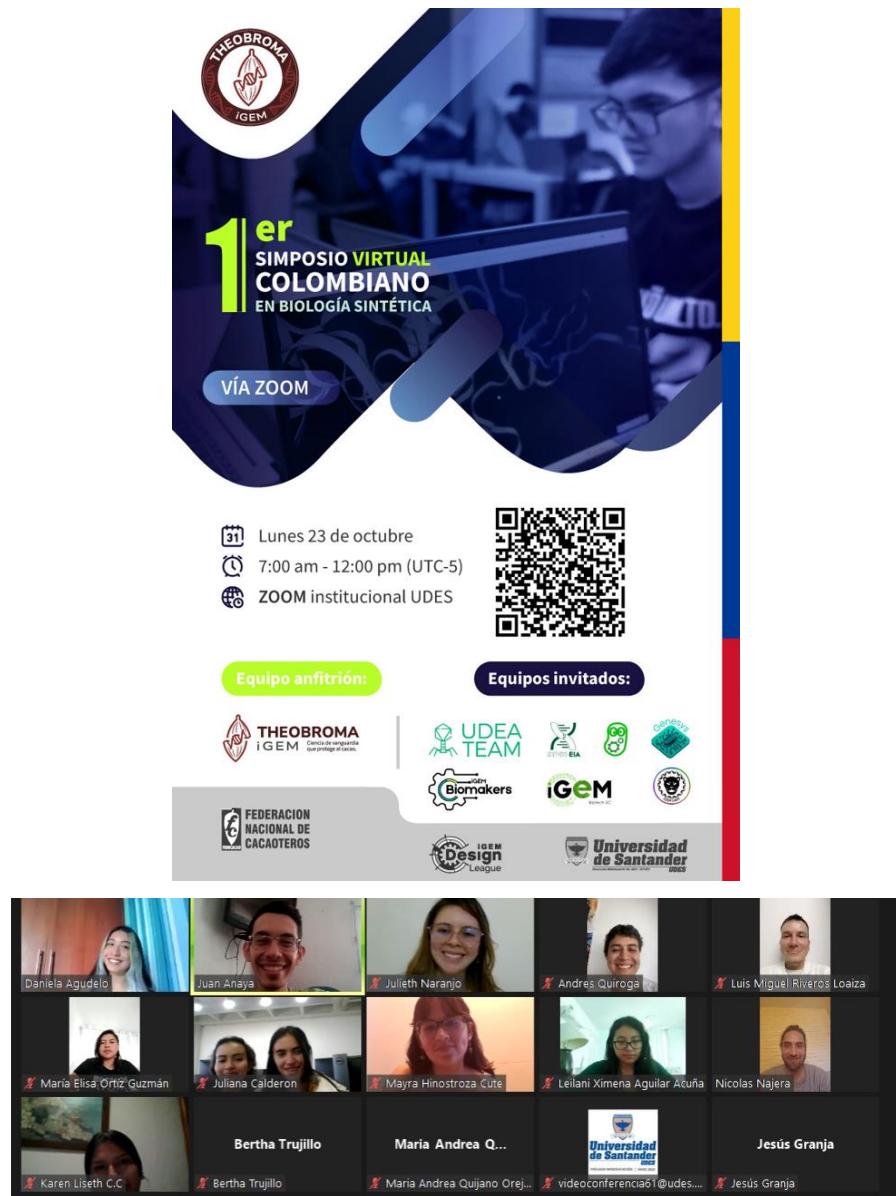


Figure 29. Colombian symposium of synthetic biology.

For more information about the symposium go to this [Link](#).

3.1.2. Partnership efforts

The most esteemed partnership of the group was formed with the igem UAM group, and this close-knit connection between the two groups originated from the shared focus on a common issue: invasive plants. From the initial interaction it became evident that a highly compelling collaboration could be established, as both groups are essentially addressing "two sides of the same coin." While the efforts of igem UAM were centered on harnessing the potential of the plant they were studying (*E. crassipes*), synthEIA was dedicated to the eradication of the invasive plant *T. alata*.

In the several meetings we stated that we would be doing some integrated campaigns regarding the awareness of the people with the problem of the invasive plants in our respective countries (Mexico/Colombia), starting with Instagram posts, stories and ideas for approaching the people in our daily lives to tell them about the problem, also finishing with a webinar about invasive plants; it was made via teams on October 14th and in which other teams (and the public in general) were invited to participate. During this webinar we first started presenting very well the problematic that not only concerns our countries but also most of the countries from Latin America, for example: we stated that these plants were introduced from other countries and rapidly started.

This partnership also made us realize that even if we could have chosen the option of choosing a specific use for the plant, it could have been dangerous for our country because people could start planting it everywhere, making it even more difficult to control its danger.

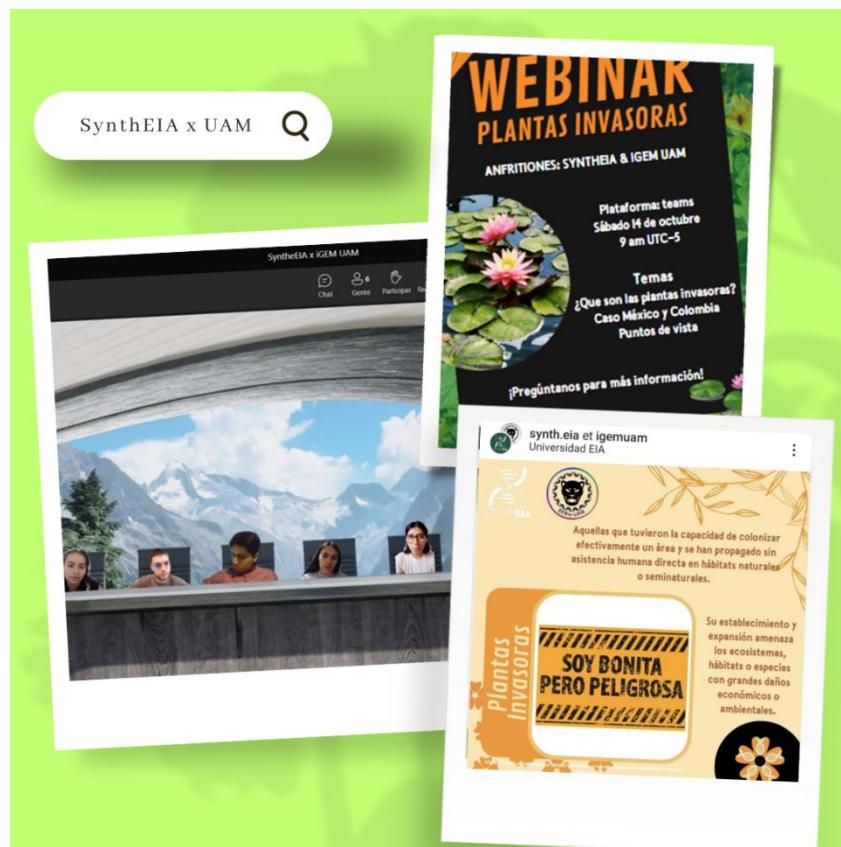


Figure 30. Partnership with igem UAM

3.2. Entrepreneurship

I. Vision, mission and objectives

Vision: To be an entrepreneurship that generates awareness on environmental conservation in Antioquia, being a reference in the biocontrol of invasive species and having articulation with governmental entities and the affected population of Antioquia.

Mission: Poeticide is a Colombian entrepreneurship that promotes research, innovation, and environmental education, promoting the control of Thunbergia alata in Antioquia through the use of a biological herbicide created from synthetic biology.

Objectives

- Launch our biological herbicide on a website with sales in the main cities of Colombia (Medellin, Bogota, Barranquilla) by 2028.
- Open the market nationwide with maximum one-week delivery by March 2030.
- To be present at environmental protection fairs and forums by March 2024.

II. Organizational Structure

Poeticide's structure has a management team that includes the areas of marketing and relations, logistics and dispatch, accounting, and finally research and development.

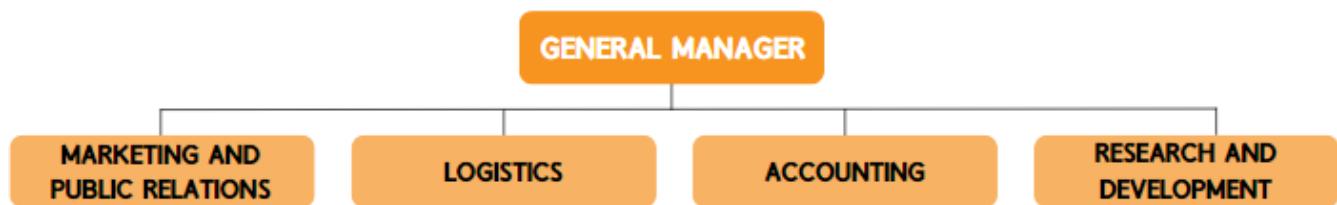


Figure 31. Organizational chart

III. Canvas model

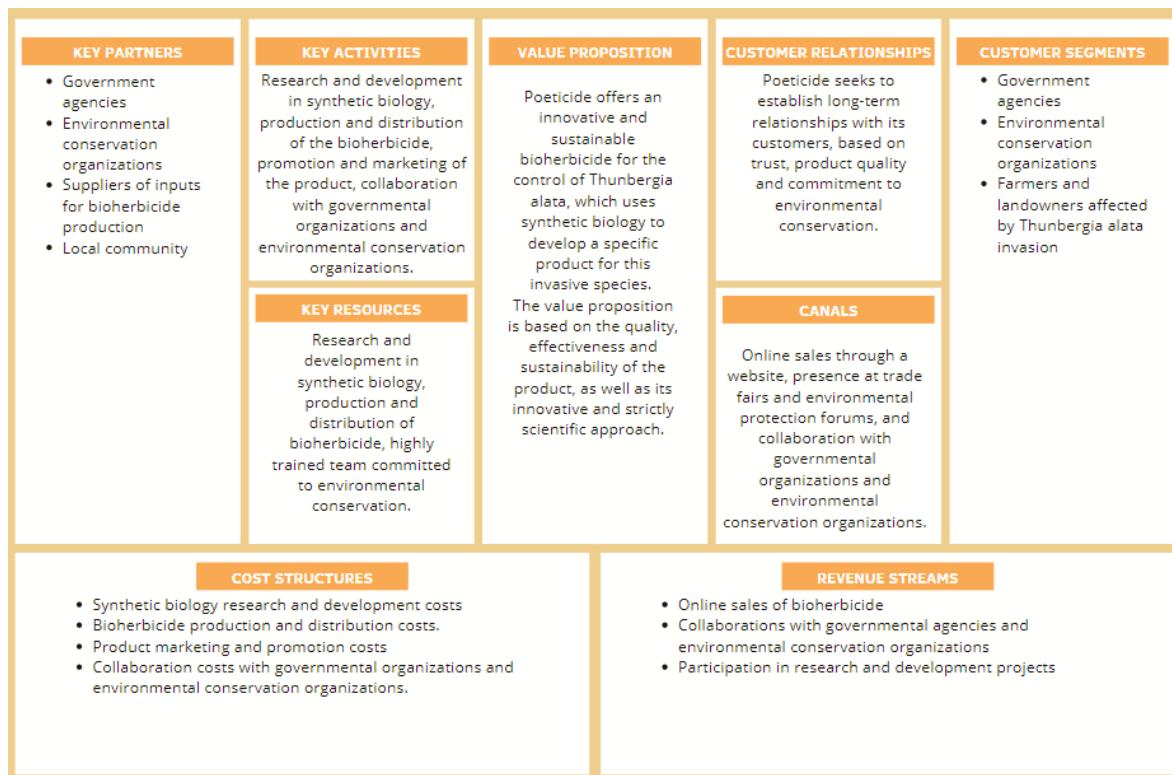


Figure 32. Canvas model.

IV. Internal analysis

Business value chain



Figure 33. Business value chain

Primary Activities

1. Supply:

Manage with suppliers the delivery of materials.

2. Internal and external logistics:

- Reception of raw material in the storage room and then inspection is performed.
- Classification and storage of raw materials.
- Provide in-house training for inventory and reporting advisers.
- Processing of customer orders, which varies depending on whether customers are:
 - Retailers
 - Wholesalers.
- Processing of payments made by customers.
- Credit management of wholesale customers.
- Management of shipments to customers

3. Research and development:

- In-depth research into the development and behavior of Thunbergia alata in Antioquia.
- Innovation in eradication processes.
- Partnerships with local and national researchers.
- Alliances with invasive plant researchers at the Latin American and international levels.

4. Marketing, sales and relationships:

- Social media management, specifically Instagram.
- Promotion and contact of new alliances.
- Participation in environmental awareness fairs and forums

Secondary activities

1. Finance:

- Management of the accounting and treasury of the company, to manage the income, expenses and other necessary accounts.
- Financial evaluation of the business line.

2. Human management:

- Recruitment and hiring of workers, mainly for the new line of business.
- Training in product handling, machinery, and customer service.
- Compensation of workers, including social benefits since all are formalized.

3. Purchases:

- Acquisition of new machinery to optimize processes.

4. After-sales service:

- Provide customer support through contact channels.
- Attention to the resolution of complaints or claims by customers.

Strategic Capabilities (Unique Resources - Tangible and Intangible)

Tangible resources

Considering that now, it is not a development that has been launched to the market and on the contrary, it is in the process of being established and developed, it is proposed to have the following tangible resources:

- Lease a facility in eastern Antioquia to be closer to the plant and the community it directly affects. This is where all the inventory would be managed, and all the logistics and research operations would be carried out.
- Inventory of all raw material to make the biological herbicide.
- Physical cell phone and computers located in the office.
- Shelving to organize the product and display them for when customers and partners visit the store.

- Own packaging.
- Personal financial support - support from banks.

Intangible resources

As described in the tangible resources' strategy, a list of intangible resources will be shown, some of which are already being developed:

- The registered trademark itself.
- The community that owns Instagram as the main social network (in development).
- The prestige or good will that the brand has in the country due to the excellent quality.
- The letter with clients and strategic alliances.
- Theoretical knowledge (in development).
- Creativity for the development of educational and inspirational content (in development).
- Organizational culture that motivates everyone inside Poeticide and is transmitted to customers (in development).
- Know how in the development of the biological herbicide (in development).

Nuclear Competences

Core Competences are fundamental skills and capabilities that an entity must possess in order to carry out its functions or activities in a particular field. These competences are essential and central to successful performance in a specific domain and are often considered as the foundation upon which other related skills and knowledge are developed. Core competences are critical to achieving objectives and goals in a given context and may vary according to the profession, industry or field in which they are applied. In the case of Poeticide, the following can be highlighted as core competences:

- It is a Colombian entrepreneurship whose production is manufactured on site, thus supporting the national and local industry.
- For the elaboration of the product, priority is given to the care of the environment and its impact on it, which makes it an environmentally friendly product that will not interfere with the ecosystem surrounding the plant.

- The aim is to have an affordable price for the community, since the company is aware that the areas affected by this invasive plant are not the areas with the greatest capital resources, therefore, by encouraging the use of our bioherbicide, we seek to make the price competitive.
- Another point to highlight is that Poeticide is born from a university initiative to have a positive impact on its environment, so social awareness is one of the main pillars of the project.

In addition, other nuclear competencies that could be developed in Poeticide to achieve greater added value in the industry could be:

- **Innovation:** the aim is to constantly evaluate the evolution of the impacted ecosystems and identify the constant improvement actions to always be delivering a product that fulfils its function, is easy to use and at an excellent price. Also, offer new environmental awareness programs and education in environmental protection.
- **Manageability:** This competence is based on the ability to manage your products, employees and other areas.

Internal Auditing Profile

Table 5. Internal Capacity Profile

	Strengths			Weaknesses			Impact		
	A	M	B	A	M	B	A	M	B
MANAGEMENT CAPACITY									
Corporate image	x						x		
Use of strategic plans and analysis		x					x		
Environmental assessment and prognosis		x					x		
Speed of response to changing conditions			x				x		
Flexibility of the organizational structure		x						x	
Habilidad Ability to attract and retain highly creative people		x					x		

Ability to respond to changing technology					x		x	
Ability to handle economic fluctuations			x			x		
Aggressiveness in dealing with competition			x			x		
Control system		x					x	
Management evaluation	x					x		
HUMAN TALENT CAPACITY								
Academic level of personnel		x					x	
Technical expertise			x			x		
Stability			x				x	
Motivation	x					x		
Ability to execute tasks	x						x	
Training	x					x		
Organizational climate	x					x		
Principles and values of the organization	x					x		
Objectives	x					x		
Knowledge of all employees of the Vision, Mission, and objectives of the company.	x						x	
Identification with the position		x				x		
COMPETITIVE CAPACITY								
Quality and exclusivity	x					x		
Customer satisfaction	x					x		
Market share	x					x		
Low distribution and sales costs			x				x	

Investment in R&D for the creation of new products				x					x	
TECHNOLOGICAL CAPACITY										
Technical and development skills					x		x			
Innovation capacity		x					x			
Level of technology used in the services	x							x		
Added value of the service	x						x			
Level of coordination and integration with other areas	x						x			
Technology application		x						x		
FINANCIAL CAPACITY										
Access to capital when required						x	x			
Ability to compete on price		x						x		
Capital investment			x				x			
Working capital	x						x			

Management capacity: with the evaluation in the previous Table , it can be observed that, although the management capacity has high strengths, there are some opportunities for improvement, essentially in terms of adaptability to environmental changes.

Human talent capacity: this is one of Poeticide's best capabilities, since the project's mission and vision are aligned with the professional and social aspirations of all its members, while providing knowledge and generating an environment of cooperation and companionship..

Competitive capacity: Since it is a unique solution in the market, it is one of the most favourable competences of this project, since it has a good relationship with potential customers, which is one of the priorities of the awareness campaigns and the improvement of the product in favour of its use.

Technological capacity: although the project is based on innovation and has a good research and laboratory team, there are only three people with professional degrees, the rest of the team are still students in training. The aim is to attract professionals to develop the project's technical capacity and share their experience. It is also necessary to purchase the equipment once it is decided to carry out the project.

Financial capacity: the seedbed of which the project is part has an available budget, but an exhaustive process of justification of expenditure must be carried out. In the same way, although the seedbed has various benefits for those who are part of it, it is not a project that generates profitability.

V. External Analysis

PESTEL Analysis

Political factors

- Governmental regulations on international trade and customs duties may affect the import and export of materials for production.
- Tax policies can affect profitability, including sales tax and income tax.
- Political and social stability in the countries where the supply chain and customers are located can influence the security and continuity of production and distribution.
- Access to funding and government programs to support entrepreneurs may also be a political factor. However, the government of the current president, Gustavo Petro, has stated that it will not continue with the orange Economy policy, which aimed to "generate decent employment in the cultural sector, support the materialization of new creative ideas and innovative products, strengthen ancestral knowledge, cultural heritage practices and the transmission of traditional knowledge" (Cedeño, 2022). However, partnerships with this sector can be chosen for social benefit.
- Environmental regulations in Colombia may influence the approval and use of bioherbicides in the control of invasive species.

Economic factors

- Biotechnology is a concept that is highly dependent on the global industry, so the exchange rate can significantly affect production costs. The currency of the country where the raw material is

produced can be devalued against other currencies, as well as the cost of production. Likewise, the import costs of the products will be more expensive, which will also significantly affect their prices.

- Consumers are expected to be on a tight budget due to economic shocks, record inflation and supply shortages, which have increased the cost of living.
- The global and national economic situation, including inflation and interest rates, may influence the company's ability to access financing and customers' consumption habits. In March 2023, the usury rate in Colombia reached one of the highest levels since 1999, at 46.26%, which affects consumer and ordinary loans such as credit cards (El Tiempo, 2023).
- Today inflation is one of the biggest challenges for many countries in the world, and according to the magazine El Portafolio, "in Colombia the trend continues to be upward and, according to analysts, it will only start to decrease until the second quarter of the year. In January 2023 the country had an inflation of 1.78% with an annual variation of 13.25%, the highest in 24 years. This makes the country the third with the highest consumer prices in Latin America" (Álvarez, 2023).

Socio-cultural factors

- Cultural and fashion trends in the care and protection of the environment from invasive plants can affect product demand.
- Education and training of customers on land and crop care techniques can affect interest and demand for yarns.
- Due to the financial crises that have occurred in recent years that have led to high levels of inflation, and consequently have affected the purchasing power of consumers, by 2023 there is a tendency to be more financially responsible, so they choose a conscious consumption (Núñez, 2023).

Technological factors

- Technology can influence the way the company produces and distributes its products. The implementation of new technologies can improve the efficiency of the production process and inventory management, which can reduce costs and improve the quality of customer service.

- The development of new production and distribution technologies can affect a company's efficiency and profitability, as well as its ability to keep up with market trends.
- Digital technologies, such as online marketing and social networks, can influence the way the company reaches its customers and promotes its products (Gil, 2022).
- Automation of production and logistics may also be a relevant technological factor to consider, as the market for robots and automation is developing at an exponential rate (AP, 2023).
- According to a report by ResearchAndMarkets.com, the global biotechnology market is expected to grow at a compound annual growth rate of 7.4% between 2021 and 2028. In addition, biotechnology has been recognized as one of the most important and fastest growing industries in the world, with a significant impact on the global economy (ResearchAndMarkets.com, 2021 & Biotechnology Innovation Organization, n.d.).

Ecological factors

- There are some regulations within the industry to promote that companies have a real commitment with the environment, within this is the ISO 14001 standard (ICONTEC, n.d.), which aims that companies are certified and acquire a real commitment with the environment, promoting an environmental management system within companies that generates economic benefits and improves environmental care.
- The impact of having an ISO 14001 certified company would be very beneficial as it would demonstrate the organization's commitment to prevent pollution and control its environmental impacts, thus promoting sustainability and care for the environment.
- Another factor to take into account is the climatic patterns that may influence the distribution and spread of *Thunbergia alata*, as well as the evaluation of the environmental impact of the bioherbicide on non-target ecosystems.

Legal factors

- The Company must comply with laws and regulations relating to intellectual property and trademark ownership. Poeticide must protect its designs and products to avoid unfair competition and unauthorized use of its creations. Thus, intellectual property in Colombia is enshrined in Article 61 of the Political Constitution of 1991; however, for the specific case of the designs and products developed by Poeticide, these would be framed within intellectual property, which is

protected from the Code of Commerce and is registered with the Superintendence of Industry and Commerce (SIC, 2017).

- The company must comply with labor and safety laws to ensure the well-being of its employees. Labor laws in Colombia are defined in the Substantive Labor Code, which establishes the remuneration, working conditions and other elements that the company must comply with to ensure compliance with workers' rights (ILO, n.d.), which may affect production costs by increasing the value of labor.

The following Table is proposed as a summary of the PESTEL analysis carried out previously.anteriormente.

Table 6. Analysis PESTEL summary

PESTEL	Threat	Oportunity	Impact		
			High	Medium	Low
Political					
Governmental international trade regulations and tariffs	x			x	
Fiscal policies	x			x	
Political and social stability	x		x		
Access to financing and government programs to support entrepreneurship.		x	x		
Environmental regulations for bioherbicide use	x			x	
Economical					
Change in exchange rate	x			x	
Product demand		x		x	

Decrease in consumer purchasing power	x		x		
Inflation and interest rates	x		x		
consumer prices	x		x		
Sociocultural					
Cultural and fashion trends towards environmental care		x	x		
More sustainable customer preferences and consumption habits		x	x		
Customer training on crop and environmental care techniques		x	x		
Changes in consumption habits due to greater financial responsibility	x		x		
Technological					
Implementation of new technologies	x	x	x		
Development of new production and distribution technologies	x			x	
Use of digital technologies such as e-commerce		x	x		
Production and logistics automation		x			x

Biotechnology boom		X		X	
Ecological					
Contamination of cotton crops	X			X	
Certification in ISO 14001 standards		X		X	
Climate patterns and environmental impact	X		X		
Legal					
Intellectual property and trademark ownership		X		X	
Labor and social security laws		X			X

Analysis of competitive forces

PORTER'S FIVE FORCES

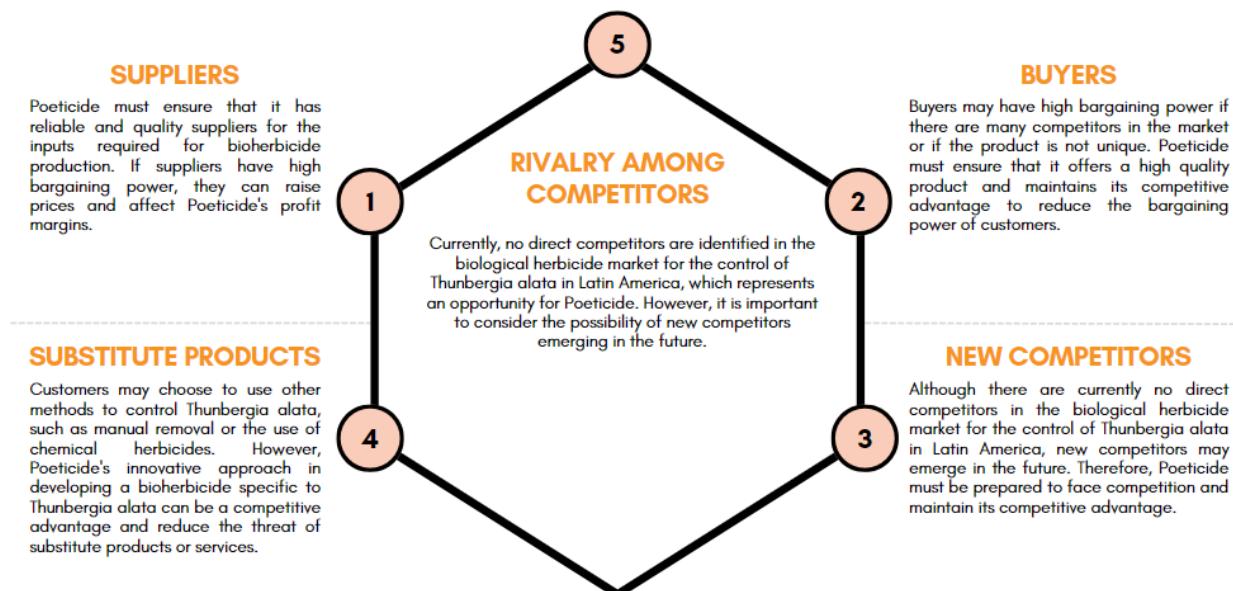


Figure 34. Porter diagram

Globally evaluating, Porter's five forces analysis indicates that there is an opportunity in the biological herbicide market for the control of *Thunbergia alata* in the Latin American region. However, it is important to consider the possibility of future competitors and maintain a competitive advantage through innovation and product quality. In addition, Poeticide must ensure that it has reliable and quality suppliers and offer a high-quality product to reduce the bargaining power of customers.

Competitive Profile Matrix

Alternative methods for *Thunbergia alata* control include manual and chemical options, as well as Poeticide's innovative alternative, which uses synthetic biology to develop a bioherbicide specific for *Thunbergia alata*. Each of these methods is briefly described below:

- a. **Manual control:** This method involves manual removal of the invasive plant by pulling out the roots and stems. While effective for controlling small populations of *Thunbergia alata*, it can be difficult and costly to implement over large areas and can be labor intensive.
- b. **Chemical herbicides:** Chemical herbicides are a common choice for control of invasive species, including *Thunbergia alata*. However, these products can have negative effects on the environment and human health, and can be costly to apply and maintain.
- c. **Poeticide:** Poeticide is an innovative alternative that uses synthetic biology to develop a bioherbicide specific for *Thunbergia alata*.

In summary, alternative methods for *Thunbergia alata* control include manual and chemical options, as well as the innovative alternative of Poeticide. Each of these methods has its advantages and disadvantages, and the choice of the most appropriate method will depend on the specific circumstances of each situation.

Key Success Factors	Weight	Manual		Chemical Herbicides		Poeticide	
		Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Quality	0.1	3	0.3	4	0.4	5	0.5
Effectiveness	0.2	2	0.6	4	0.8	5	1
Environment	0.3	5	1.5	2	0.6	5	1.5
Adaptability	0.2	2	0.4	3	0.6	4	0.8
Innovation	0.1	1	0.1	2	0.2	5	0.5
Costs	0.1	5	0.5	3	0.3	4	0.4
TOTAL	100%		3.4		2.9		4.7

Figure 35. Competitive Profile Matrix for Poeticide

In the matrix, it can be seen that Poeticide scores the highest on most factors, indicating that it is a more favorable alternative for Thunbergia alata control compared to the other two options. Poeticide stands out for its high quality, effectiveness and innovative approach in developing a bioherbicide specifically for Thunbergia alata. In addition, Poeticide scores high on the environmental factor, indicating that its use is more sustainable and environmentally friendly compared to the other two options. In terms of cost, Poeticide scores intermediate, indicating that its cost may be slightly higher than manual control, but lower than chemical herbicides. Finally, Poeticide scores highest on the adaptability factor, indicating that it is a more flexible and adaptable alternative to different situations and conditions.

Stakeholder Matrix

First of all, a list of Poeticide's stakeholders and their respective interests is developed; the following is obtained:

Table 7. Relationship between stakeholders and interests

Stakeholders	Interest
Local community	<ul style="list-style-type: none"> - Biodiversity and local ecosystem conservation - Reducing the environmental impact of <i>Thunbergia alata</i> - Generation of employment and local economic development
Government endorsements	<ul style="list-style-type: none"> - Compliance with environmental regulations - Promotion of environmental conservation and sustainability - Economic development and job creation
Customers	<ul style="list-style-type: none"> - Effective control of <i>Thunbergia alata</i> - Sustainability and environmental friendliness - Product quality and efficacy
Suppliers	<ul style="list-style-type: none"> - Stable and reliable business relationship - Quality and consistency of inputs
Investors	<ul style="list-style-type: none"> - Profitability and return on investment - Potential for business growth and expansion - Positive social and environmental impact
Work team	<ul style="list-style-type: none"> - Safe and healthy work environment - Opportunities for professional growth and development - Contribution to the solution of an important environmental problem.

In summary, the stakeholder matrix for Poeticide identifies the relevant stakeholders and their interests in relation to the business idea. The local community and governmental bodies are interested in environmental conservation and local economic development, while customers seek effective control of *Thunbergia alata* and a sustainable, high quality product. Suppliers seek a stable and reliable business

relationship, while investors seek profitability and a positive social and environmental impact. The work team seeks a safe and healthy work environment, as well as opportunities for professional growth and development. It is important that Poeticide takes these interests into account and works in collaboration with its stakeholders to achieve a positive impact on the community and the environment.

Subsequently, the relationship with each of them and their interests are analyzed to determine the group to which they belong and diagram it in the figure. Likewise, the results obtained in the previous matrix analysis that provided results regarding the power that the agents have in the company are taken into account.

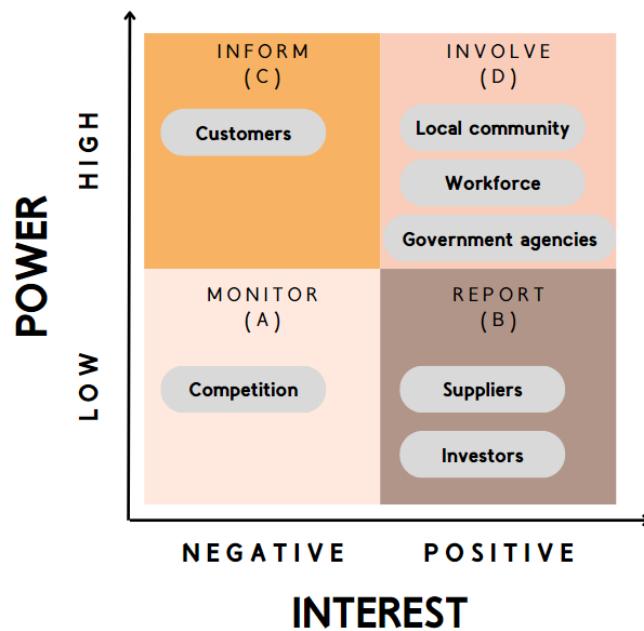


Figure 36. Stakeholders matrix

Finally, the conformation of the groups and the reasons that determined the classification are explained. All this according to the type of relationship they would have with the project:

- **Group A (monitor):** In this group we classify the competition, since the level of rivalry is rated as low; thus, it is categorized as a group with little power and low interest. However, as they are members of the market in which the company operates, regular monitoring and follow-up of their movements and actions should be carried out to be aware of new trends or innovations that may arise that represent a threat or an opportunity.
- **Group B (Reporting):** Suppliers and investors were classified in this group, since they are important for the operation of the company, in terms of alliances, prices and influences in the environment.

Therefore, it is considered that these stakeholders have great interest and need to be informed about the evolution of the project.

- **Group C (Inform):** Customers are classified here, since this group needs to be constantly informed about the company's movements, such as new launches, price changes, and other news that may arise. They are agents that fit this definition since, although they have power, their level of interest in the organization's strategies is low, i.e., they are not directly affected by organizational decisions and are therefore not interested in them. However, they can be transferred to group D in the event of a relevant event occurring.
- **Group D (Involve):** This group includes the local community, the work team and the governmental entities, which are powerful agents with a high interest in the organization's strategies. The local community is the project's partner, so constant communication and collaboration with them is necessary for the project's success. On the other hand, the work team is directly affected by business decisions, so there is no doubt at any time about the relevance of being involved in the process. Finally, on the government side, not only the regulations and other legal characteristics that must be complied with are taken into account, but also a way of integrating into the activities offered to the communities through the different entities is sought.

SWOT matrix

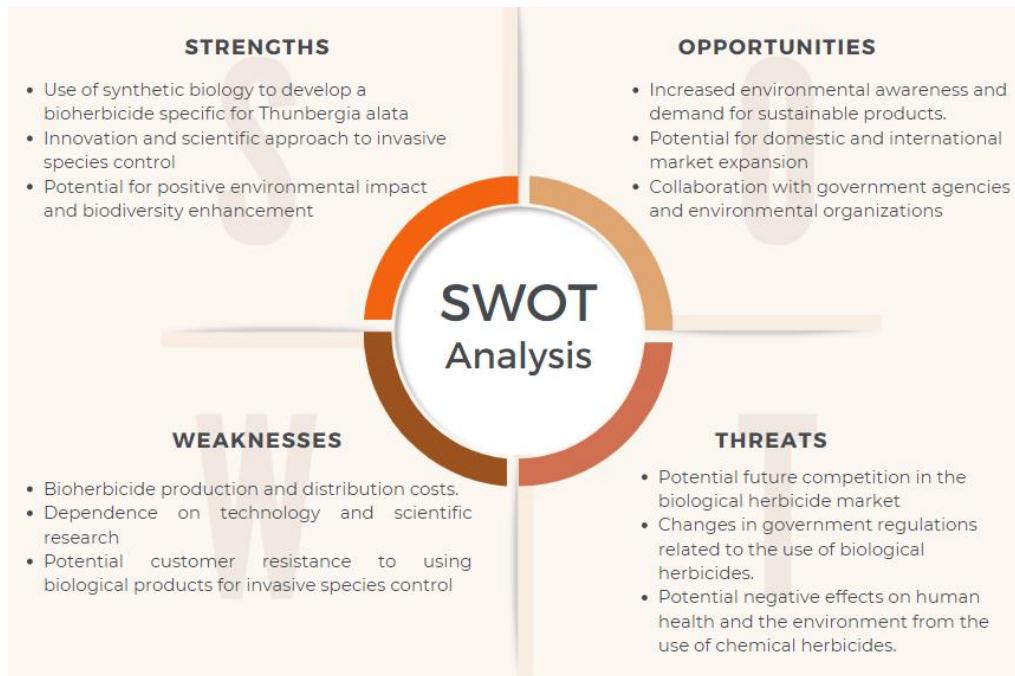


Figure 37. SWOT matrix

Empathy Map

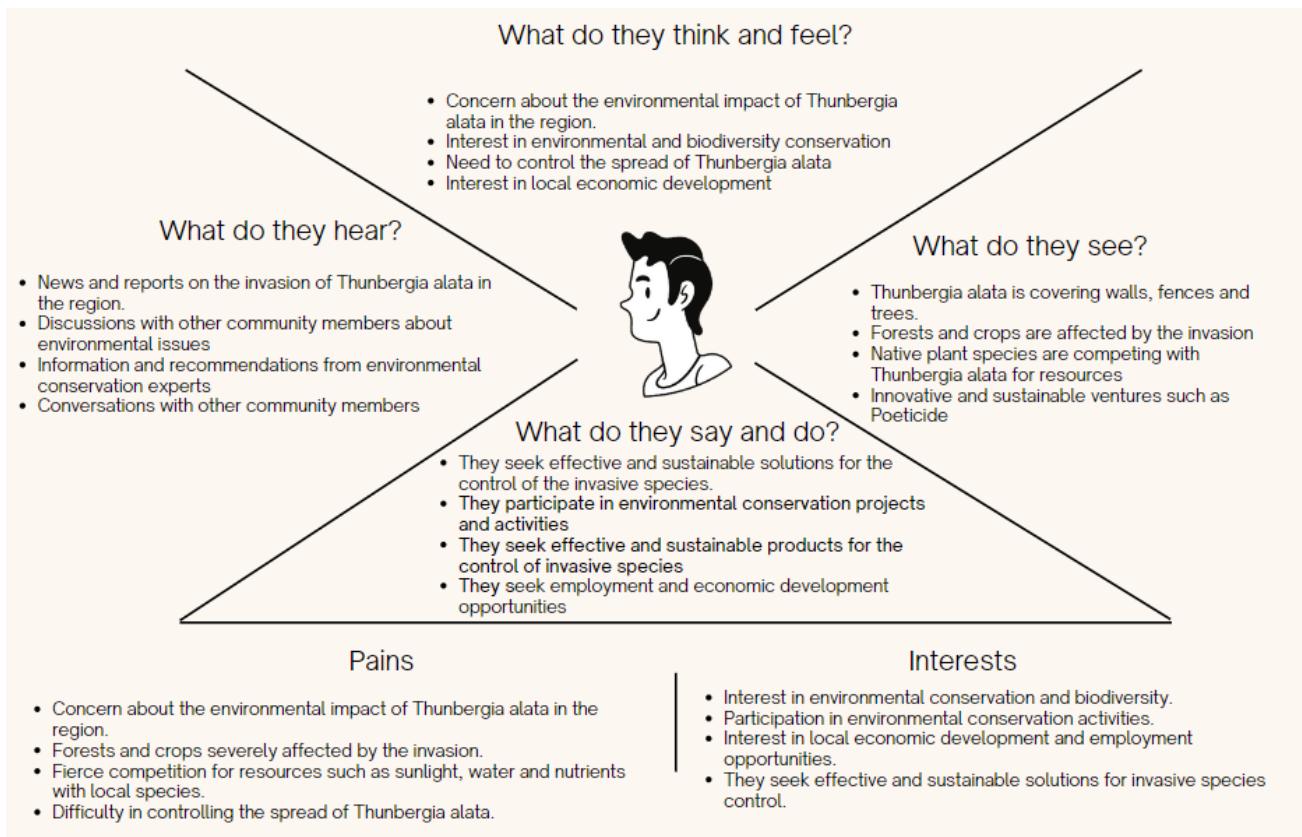


Figure 38. Empathy Map

VI. Conclusions

1. The invasion of *Thunbergia alata* in eastern Antioquia, Colombia, is an important environmental problem that affects biodiversity and the stability of local ecosystems.
2. Poeticide is an innovative venture idea that uses synthetic biology to develop a bioherbicide specific for *Thunbergia alata*, which represents a sustainable and scientifically supported solution for the control of this invasive species.
3. Poeticide has the potential to raise awareness about environmental conservation in Antioquia and become a reference in the biocontrol of invasive species, in coordination with governmental agencies and the affected population of Antioquia.
4. Poeticide's objectives include launching its biological herbicide on a web portal with sales in the main cities of Colombia, opening the market at the national level and having a presence at environmental protection fairs and forums.

VII. Recommendations

1. Poeticide should continue its research and development in synthetic biology to improve the effectiveness and sustainability of its bioherbicide and remain at the forefront of invasive species control.
2. Poeticide should establish strategic alliances with governmental organizations and environmental conservation companies to promote its value proposition and achieve a positive impact on environmental conservation and sustainability.

3.3. Science communication

The team has maintained a constant commitment in the process of science communication. We were able to do a lot of activities and share our knowledge about synthetic biology to the community and propose our solution to a local problem. These efforts involve visits to schools, including La Enseñanza, Leonardo da Vinci School, and two others in San Vicente Ferrer. Our team used the "eye of the poet" as a real-world example to illustrate the importance of an agricultural issue and its regional impact, encouraging students to explore the "Poeticide" solution. Engaging activities such as creating murals and games were used in the schools to help students to get a better understanding of the "eye of the poet" and we also provided incentives for their effort.

Within EIA University, we promote an understanding of synthetic biology among non-biology students and encourage them to contribute by avoiding the use of the "eye of the poet" plant. Additionally, the SynthEIA team is going to participate in national events like EXPOIngeniería 2023, showcasing interactive activities related to synthetic biology. An Instagram account, @Synth.eia, has been created to share information about synthetic biology, the project, and various activities. Furthermore, a visit to the villages of Jardín and Marinilla allowed us for discussions with residents and sharing information on the local issue and the "Poeticide" solution through a detailed poster.

3.3.1. Inreach efforts: iGEM Design League poster



Local problem statement

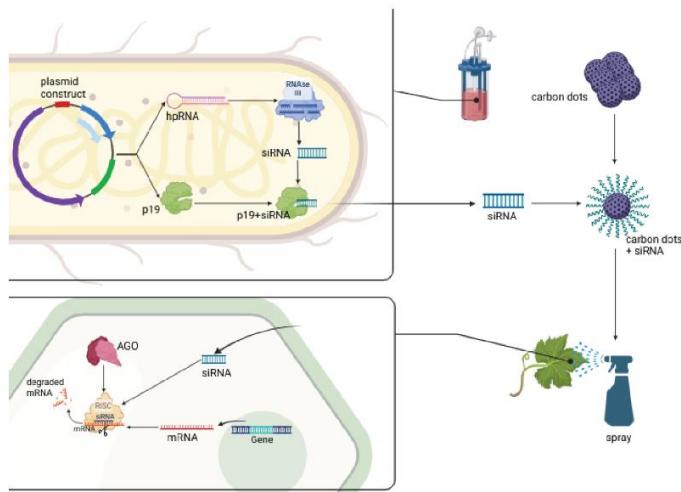
Biological invasions are one of the main reasons of biodiversity loss worldwide. *Thunbergia alata* represents one of the most problematic invasive plant species in Colombia due to its great propagation capacity, dispersal mechanisms, and accelerated development.



It has managed to colonize Eastern Antioquia, generating negative effects on the biodiversity and the production systems, causing loss in productivity and harvesting of crops. Some of its main effects are blocking the reception of solar radiation and nutrients to native species, increasing the fires, and the presence of pathogens to the fauna.

Methodology

Development of recombinant siRNAs in order to silence specific key genes of *T. alata*: FATA, FATB1, FATB2 and KASII, conjugating them to carbon dots nanoparticles that will be sprayed, facilitating their incorporation to the cells and guarantying its integrity.



Genetic circuit design

Insertion of segments of 250 base pairs in the sense and antisense directions containing a constitutive promoter (*tac*) for production of a hairpin RNA (hpRNA). A CDS will be inserted for p19 protein with a constitutive promoter (*I7*). Specific terminators for the polymerase of *E. coli* will be used in addition to an optimal plasmid for high levels of transcription in this microorganism.

Build

The siRNAs will be produced by constructing a plasmid in *E. coli* containing the specific sites for the genes to be silenced as well as the CDS of the p19 protein essential for stability of the generated siRNAs. Then, positively charged carbon dots will be produced to allow the adhesion of the siRNAs, and they will be applied by spraying them at low pressures to achieve high permeability rates in *T. alata* cells.

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Results

The expected results are as follows:

- siRNAs of approximately 21 nt in length, specific for various sites in a range of 200 to 300 base pairs of the target genes to be silenced.
- Small-sized nanoparticles produced from positively charged polyethenimine, demonstrating proper binding to the siRNAs.
- Low transcription rates of silenced genes with decreases in mRNA levels greater than 70 %.



Human Practices

Approach to the community and stakeholders; communication with government entities, environmental authorities and research groups; scientific dissemination and environmental awareness in educational institutions and towns of Eastern Antioquia; promotion of science in social networks, fairs and congresses.

Biosafety, Biosecurity & Policies

Biosafety and biosecurity in Colombia are essential components in the protection of public health and environment. We have adopted a number of regulations, such as the Cartagena and the Nagoya - Kuala Lumpur Protocols. Our proposal guarantees effectiveness, selectivity and safety.

Arts

Flowers in Antioquia are art and culture, we seek to create awareness beyond their appearance: photography and sculpture contest; murals; video game; artificial intelligence. Make people build with their hands so they learn by doing.

Test

- Anion - exchange HPLC + NanoDrop for purification of 21 nt siRNA.
- Size - exclusion chromatography equipped with spectrophotometry.
- RT-qPCR for quantification of transcript levels.

3.3.2. Outreach efforts

Schools:

A constant commitment has been maintained in the field of science communication with respect to the dissemination of science in educational institutions. The main objective is to stimulate and increase the student's interest in science training. In addition, the aim is to provide these students with a concrete vision of synthetic biology through contextualization with a real example, such as the black-eyed susan vine, allowing them to understand the magnitude of this challenge and its impact on the region. So, the intention is to motivate students to explore the solution offered by "Poeticide" and to recognize a practical use of synthetic biology.

The planned visits were carried out in several schools, including Colegio de la Compañía de María La Enseñanza, Escuela Leonardo da Vinci School.



Figure 39. Conference

During the visit to the Colegio de la Compañía de María La Enseñanza, a comprehensive conference on synthetic biology was given, in which the *T. alata* problem and the "Poeticide" solution project were addressed, as illustrated in Figure 39. Additionally, two activities designed to promote a deep understanding of the black-eyed susan vine were carried out. The first activity consisted of the creation of a handmade mural by the students, using black and orange colors, which are representative of the *Thunbergia alata* plant. Also, a game called "Black-eyed Susan vine and Eradicators" was organized, as shown in Figure 40, in which students had to collect orange ribbons that were attached to each participant, symbolizing the task of uprooting the *T. alata* plants and the person with the most ribbons plucked (flowers) was named the winner.

To motivate the students, prizes were distributed to participants and winners, such as bottles, glider books, pens, and bookmarks.



Figure 40. Game "Black-eyed Susan vine and Eradicators"

In the other three schools mentioned, the same methodology of presenting the conference on synthetic biology, the *T. talata*'s problem and the "Poeticide" solution was followed, as illustrated in Figure 41. In addition, another incentive was provided to ensure greater retention of information related to the black-eyed Susan problem. Students received bookmarks with a design developed by SynthEIA, displaying two sides, one focused on synthetic biology and the other on the *T. talata* project. Pens with the colors representing the plant were also distributed.



Figure 41. Second conference

Universidad EIA-Emprending:

From the internal diffusion within the EIA University, to which the participating team belongs, activities were carried out with the purpose of promoting the understanding of synthetic biology among the careers not related to biology. In addition, information about the project was shared, so students could identify the problem and contribute from their homes avoiding the use of the black-eyed Susan vine plant as an ornamental plant. One of these activities was the participation in the "Emprending" event, where some students presented entrepreneurship projects. On this occasion, the team produced a poster (Figure 42) detailing all relevant information about the project, with the purpose of exposing it to students, teachers, parents, and other members of the institution, to publicize the Poeticide project (Figure 43).



Figure 42. Emprending poster



Figure 43. Project presentation in “Emprending”

EXPOingeniería 2023

The national event that brings together all the engineers of Colombia will be held from November 2 to 4 in Plaza Mayor, Medellin. EXPOEngineering 2023 will be a comprehensive meeting of the system of science, technology, and innovation, emphasizing its applications in the real environment of the country. The central theme of this event is "Engineering for Transformation", with sub-themes focused on energy, infrastructure, 4.0 technology and vital engineering. The SynthEIA team of the EIA University, which is a partner of the event, had the opportunity of a stand at the innovation and entrepreneurship fair. At this stand there are demonstrations and interactive activities related to various topics, highlighting the achievements in synthetic biology research of the project.

Instagram:

To successfully communicate science, share interesting information and reach a very large audience, it was very important for the group to have an Instagram account (Figure 44). Through this platform, the group could share each member's presentation, elaborate on the meaning behind synthetic biology,

debunk associated myths, discuss the central dogma of biology, address the problematics being addressed, and explore genetic silencing via iRNAs. On the other hand, stories about the activities carried out during the competition, such as collaborations, contests, school visits, town visits, expert interviews, webinars, and many others, have also been shared on the account.



Figure 44. Instagram @synth.eia

Youtube:

An additional crucial platform utilized for widespread dissemination of project information is YouTube. This platform was employed to share important videos, including the project's promotional video, Poeticide. Moreover, it featured collaborations and partnerships with other groups, such as the webinar conducted with iGEM UAM and the Virtual Colombian Symposium in Synthetic Biology.

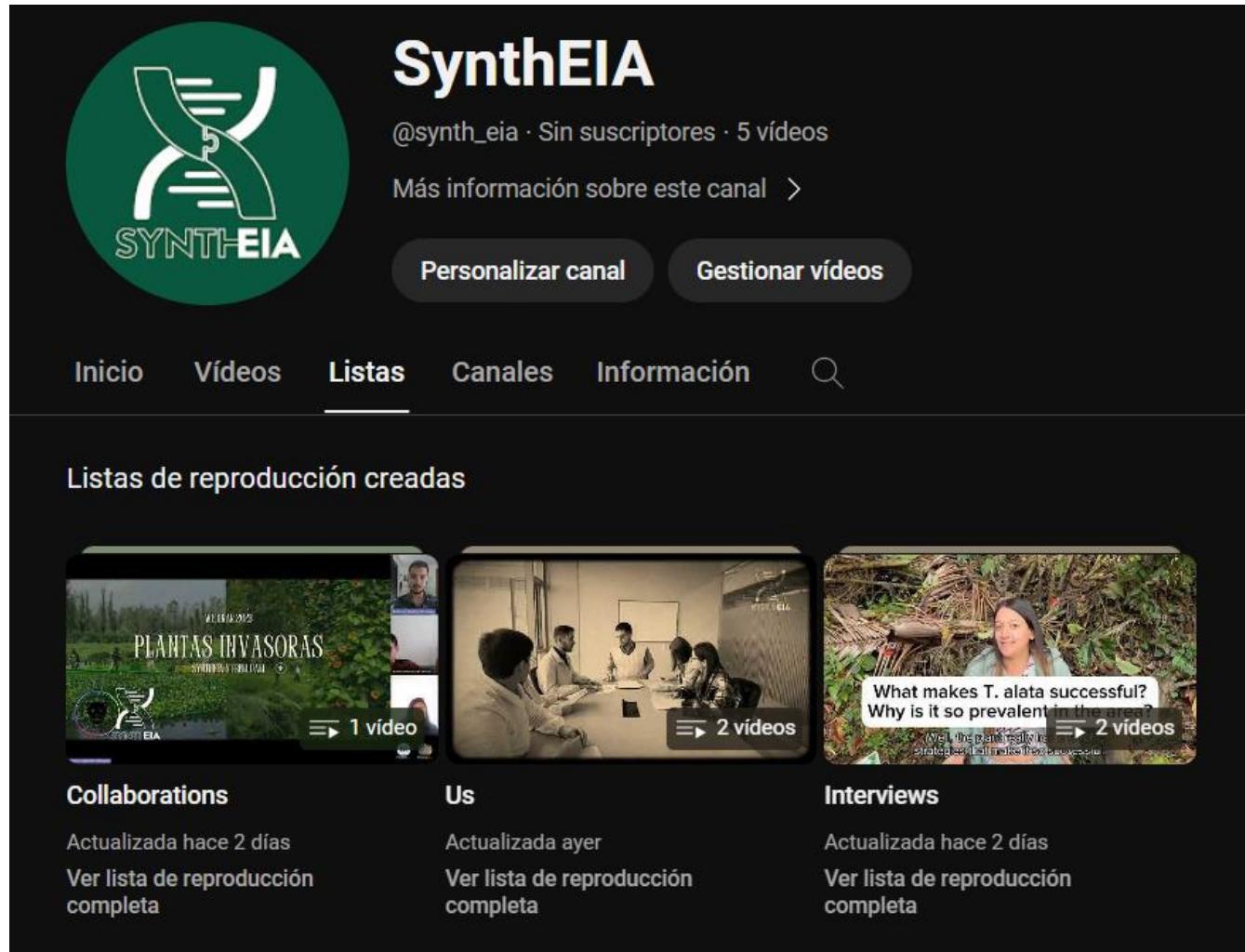


Figure 45. Youtube channel

Villages:

It was possible to connect with places that are further from the city and the university, just like Marinilla and Jardin, villages in which it was possible to do some awareness campaigns about the local problem to the residents of the zone. Some of them were not even concerned about the problem or even conscious of its seriousness, so it was very important to show them the real situation with this beautiful invasive plant.

3.4. Public Policy and Regulation

Today, it is possible to say that Colombia adopted the Cartagena Protocol on Biosafety of the Convention on Biological Diversity under Law 740 of 2002, and the Nagoya-Kuala Lumpur Protocol on Liability and Supplementary Compensation to the Cartagena Protocol under Law 1926 of 2018. It is also true that in this country there are several state organizations that regulate aspects such as biosafety, bioethics, and transportation of biologics. However, none of the above has an emphasis on synthetic biology; in fact, regulations in this regard are slightly behind other countries. Therefore, there can be a proposal to introduce various initiatives aimed at enhancing the advancement of synthetic biology within the country:

A reform of the secondary education curriculum: In the context of public education in Colombia, most schools generally do not place a strong emphasis on subjects related to biology and life sciences. This situation causes young people to leave without much knowledge of the potential of the scientific world of biology, hence, there is a suggestion to develop a legislation that fosters the comprehension of biology and its related fields in secondary education, aiming to encourage critical thinking, scientific literacy, and the preparation of future citizens aware of scientific challenges and developments.

To accomplish this goal, the following is recommended:

- Develop a new curriculum that includes biology and related science subjects in the secondary school. This could include molecular biology, genetics, ecology, botany, zoology, microbiology and other related disciplines.
- Train teachers to be ready to teach these new subjects. Professional development opportunities should be offered to update their knowledge and skills.
- Provide schools with teaching materials, laboratories, textbooks and access to online resources to support the teaching of new subjects.
- Design effective assessments to measure student learning in biology and related sciences. This includes standardized tests and hands-on assessments.
- Encourage student participation in extracurricular science activities, such as science fairs, science clubs, and research projects, through incentives that will help them in their future university careers.
- Establish links with local scientific institutions and universities to provide students with hands-on experiences and real-world learning opportunities.

- Regularly assess and adjust the implementation of educational reform to ensure that it certainly is meeting its objectives and improving science education.
- Ensure an adequate budget to implement these changes, including the teacher training, the acquisition of resources and the updating of the curriculum. Also, establish a clear timeTable for the implementation of the reform, dividing phases into academic years and monitoring progress.

With the implementation of this project, an improvement in scientific literacy is expected, where high school students gain a deeper understanding of key scientific concepts, enabling them to make informed decisions about scientific and technological issues in their daily lives that impact their future and that of the country. Moreover, the promotion of critical thinking will further develop skills such as problem solving and the ability to question and analyze information objectively.

Benefits will also be reflected in Colombian higher education, as students will enter their careers better prepared because by exposing students to scientific concepts from an early age, interest in research and innovation can be stimulated, which will strengthen life science programs and the country's scientific muscle; as it prepares students for future careers in scientific, technological, medical and research fields, thereby increasing employment and economic opportunities.

Providing all students with the opportunity to access a solid science education can contribute to reducing inequalities in access to education and employment opportunities. A more science-educated workforce can drive innovation and competitiveness at the national level, which is essential in today's global economy. The political landscape and debates surrounding Colombian society will gain strength in arguments as students will be better prepared to understand and participate in political debates and decisions related to scientific and health issues. Hand in hand with this, scientific research is fostered at the local level and collaboration between academic institutions and industry is promoted, strengthening diverse fields of scientists such as biotechnology, synthetic biology, genetic engineering, bioinformatics, etc.

With this responsibility and proposal in mind, SynthEIA has contributed a bit by visiting schools to promote the early development of scientific skills and to raise awareness of the universe of possibilities offered by the biological sciences for the future and the present. The group is hoping that this participation in the competition will inspire the youngest to design with biology.

3.5. Arts

Initially, a survey of the target population was carried out and it became evident that there was a high rate of ignorance about the invasive species *Thunbergia alata* (black-eyed Susan vine) and what it truly meant to have it in the ecosystem, as well as the scope of biotechnology and synthetic biology to solve this type of problem.

In view of this, the group developed different strategies, like the ones mentioned below, that would bring the community closer to these topics and would be easy for them to understand, even if they did not have specialized formation:

I. Photography and sculpture contest

A contest was held in which the community was invited to capture the reality of the problem, thus promoting awareness and reflection on the importance of addressing this ecological challenge. The general public could participate by submitting evidence of a photograph or sculpture reflecting the problem of the invasive species *Thunbergia alata* that plagues the entire region.

For this it was necessary a close cooperation with the social media team, to make publications about it on Instagram, with the science communication team for them to notify the people present at their conferences and encourage them to participate, and lastly with the collaboration team who shared the information on Discord.

These efforts allowed the participation of students from local universities, different schools, the iGEM community and opened the way to a greater knowledge of the problem in a dynamic and competitive way. In this [link](#) you can find the pictures participating in the contest.



Figure 46. Photography contest

II. Poeticide Video Game

The team developed an interactive video game featuring the problematic, black-eyed susan vine, to raise awareness of the invasive population while providing a playful understanding of the potential positive impact of synthetic biology in solving environmental problems. You can access the game in [this link](#).

The game follows a model similar to MarioBros, however, it was created in an environment full of trees and flowers of two types, in order to advance and reach the goal without losing because of the invasive species. With this, it was sought to show the community in a didactic way, that, although the eye of a poet is a plant whose flower is very striking, it must be eradicated because it causes damage to the species around it. The game can also encourage competitiveness by playing with a set time and reaching the goal before it runs out, or playing so that each eradicated flower is a point and wins who has the most points.

In this way, awareness of the problem is generated in a fun space, which was shared with other teams of the competition (collaboration) and with the students of the schools (communication of science).

III. Cultural exhibitions

cultural exhibitions were created, such as traditional Silletas, which represent the Colombian identity. They were used as visual means to illustrate in an impactful and friendly way the harmful effect of the invasive plant on the local ecosystem and how our synthetic biology proposal can offer an effective and sustainable solution. Attached below are photos of the project's Silleta made by SynthEIA team:



Figure 47. Poeticide's Silleta

IV. Murals

After the conferences in the schools there was a space for creating murals where a visual synopsis of the topics exposed was represented; such as synthetic biology, biotechnology and *T. Talata* (invasive species). As viewed in Figure 48.



Figure 48. Mural made by school students

With these strategies it was possible for the group to consolidate the knowledge about the problematic and synthetic biology. Likewise, there was a solid approach with the community, for example, in the photography contest it was possible to create a relationship with the artistic community, in which 50% of the participants were students at the EIA University, 12% were collaborators (teachers, people of various services, administrative personnel) and 37.5% were relatives of the EIA students. Also, on the part of the video game, 207 visits were obtained in a month, of which 20% were related to the EIA university and the remaining percentage corresponded to the external community, such as iGEM participants, students from different universities and people from the region.

The cultural exhibitions managed to create strong ties with the university students and the virtual community, with hashtags such as #BonitaBeroPeligrosa (BeautifulButDangerous) from the initial publications of the saddle (silleta). Finally, a strong relationship was created with the schools and, even more importantly, with their students, who, upon finishing their murals, felt an affinity with the themes addressed.

However, the main intention was to increase public awareness regarding the issue of the invasive plant *T. Talata* in the region through interactive tools, activities and educational games; for this, it was necessary

the implementation of a characteristic image design that would allow people to deeply understand the problem through a visual and artistic demonstration of the things that were intended to spread. Through social media, the color palette was chosen directly in representation of the flower, so the posts uploaded have been in orange and green, a very meaningful approach to capture the attention of the people, that is pretty much needed in this awareness context.

V. Graphic manuals

Image manuals were designed for both the project and the research group as a guide for social network management, science communication, among other activities. The manuals can be accessed in this [Link](#).



Figure 49. Graphic manuals