DESIGN

The work begins starting with the design of the bioparts

The development of whole-cell biosensors for heavy metals is widely described in the literature, within the iGEM competition many teams have explored various well-established design approaches. In an attempt to differentiate ourselves our team has decided to take a less explored approach for the design of our arsenic biosensor, based initalt on the work of Wang et al. (1) It relies mainly on 3 strategies:

I. Control of intracellular density of arsR protein

Normally arsR regulates its own expression within the arsRDABC operon (2) and in whole-cell biosensors with a more conventional design, recent studies have been conducted where the expression of arsR has been placed under the control of constitutive promoters of different strengths, this has resulted in a finer and more comprehensive control of outputs. (3)

A weak constitutive promoter controlling arsR expression results in a lower concentration of arsR protein in the bacterial cytosol, loosening the control of the expression that it exerts and the minimum concentration of arsenic necessary to activate the genetic constructs, in other words, the detection limit of the biosensor is improved. On the other hand, if an arsenic biosensor with a sensitivity for higher concentrations is required, the promoter can be changed to one of greater strength.

Figure 1. Adjusting the intracellular receptor concentration allows manipulating the detection limit of the biosensor. a) Strong constitutive promoter: higher concentration of ArsR results in a higher concentration of Arsenic for the activation of the constructs. b) Weak constitutive promoter: lower ArsR concentration results in a lower Arsenic concentration for the activation of the constructs.

II Using simple and cascading transcriptional amplifiers

Upon activation of the constructs, the transcriptional signal generated will be received by the HrpRS and/or RinA amplifier systems and will be scaled by a gain factor to produce an enhanced output signal. Being able to predictably scale a transcriptional signal provides a new level of control and flexibility over the outputs of genetic constructs where low-level or saturated transcription signals must be scaled to increase sensitivity. Like its electronic counterparts, amplifier engineering is critically valuable in customizing signal processing in cells for various applications. (4)

HrpRS system : it is composed by the genetic components (hrpRS and PhrpL) of the regulatory network of the hrp gene (hypersensitive reaction and pathogenicity) for type III secretion in Pseudomonas syringae . Activating hrpR and hrpS proteins results in the formation of an ultrasensitive complex which binds a sequence up-stream the hrpL promoter (σ54 factor dependent) for remodeling the closed transcription σ54-RNAP-hrpL complex into a open one through ATP hydrolysis for finally promoting transcription. (5)

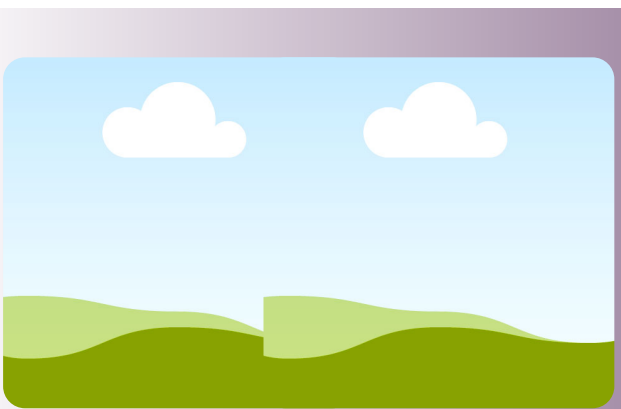


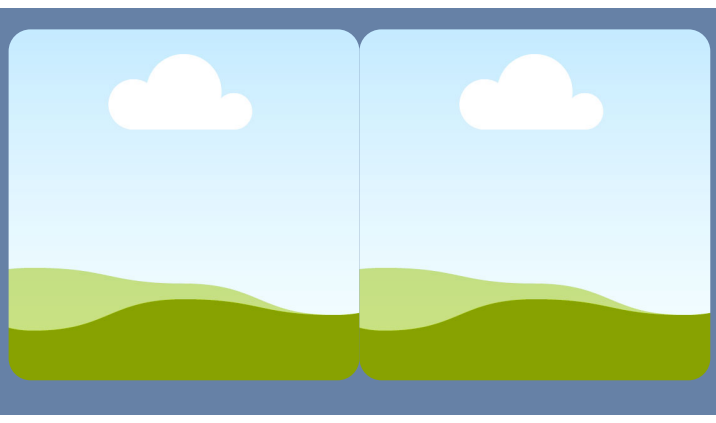
Figure 2. The HrpR and HrpS proteins form a hexameric complex that functions as an enhancer element that comes into contact with RNA polymerase and σ54 factor, which in turn are positioned on the PhrpL promoter, activating transcription in an ultra-sensitive and amplified way.

RinA system : consists of a family of phage-encoded proteins that act as activators for the transcription of late operons in a large group of Staphylococcus aureus phages . It has the ability to bind to the PrinA_p80 promoter sequence where it promotes transcription of down-stream elements in an ultrasensitive way. (6)

III Chromoprotein as a reporter

After processing the transcriptional signal, it is necessary to convert it to a measurable type of signal, in our case this is possible through the use of a reporter gene that produces a colored protein observable to the naked eye. Chromoproteins have certain advantages over other fluorescent proteins, such as having dark colors under ambient light that allow inexpensive analysis without specialized instruments. They also avoid problems due to background fluorescence, UV-induced bleaching, cell damage, and the need for eye and skin protection. Applications of chromoproteins include markers in living organisms for cloning (7), teaching (8) and biosensors. (9)

Figure 3



mRFP1E Chromoprotein: its a variant of the monomeric red fluorescent protein 1 (mRFP1) gene that was codon optimized for E. coli (abbreviated mRFP1E). It produces a dark red color observable to the naked eye and has a fluorescent capacity with an excitation spectrum at 582 nm and an emission spectrum at 606 nm. Is low molecular weight (55 kDa) and it has been reported that its toxicity on E. coli is considerably lower than other chromoproteins, which is a great advantage for its use as a reporter, its maturation time is 18 - 24 hours in the presence of oxygen. Orange, pink, magenta and violet color variants have also been generated with the same characteristics (10)

Red fluorescent proteins such as mRFP1 have an absorption spectrum that can overlap with optical density measurements of cultures at 600 nm (OD600), causing interference and overestimations.111 For this reason we have opted for the violet variant of this chromoprotein, mRFP1-Violet , its absorption spectrum is in the region of 350 - 450 nm, eliminating the interferences with OD600. It has been generated by mutations of the original chromoprotein fluorophore site and retains its characteristics such as fluorescence, maturation time and low toxicity. (10)

ARSENITO IN DETAIL

We apply the aforementioned strategies to generate 4 genetic constructs that offer different ranges of sensitivity to arsenic. Transformed into 4 different E. coli BL21 strains, we can generate traffic light patterns visible to the naked eye and easy to interpret.

Figure 4. The different arsenic sensitivities of the constructs allow semi-quantitative interpretation of results by means of traffic light patterns observable with the naked eye.

1. As0 Construct (≥ 0.5 ppb [As] sensitivity):

The detection module consists of a weak constitutive promoter (BBa_J23109) that is in charge of the expression of the arsR protein (BBa_J15101) , next is the processing module that is made up by Pars inducible promoter (BBa_K190015) , which receives the transcriptional signal, and a double amplifying cascade (HrpRS-pHrpL-RinA), finally is the output module made up by an inducible promoter pRinA-p80 that receives the amplified signal of the cascade and promotes the expression of the reporter gene mRFP1 -Violet.

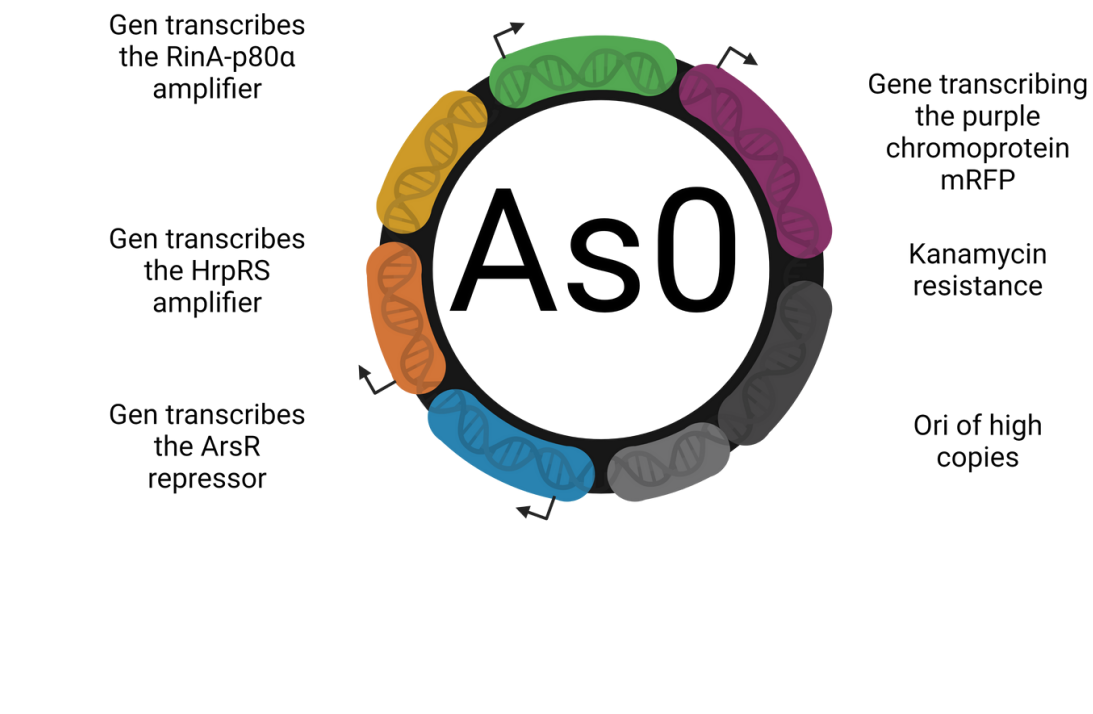


Figure 5.

2. As2 Construct(≥ 3 ppb [As] Sensitivity):

The detection module consists of a weak constitutive promoter (BBa_J23109) that is in charge of the expression of the arsR protein (BBa_J15101), next is the processing module that is made up by Pars inducible promoter (BBa_K190015) , which receives the transcriptional signal, and a simple transcriptional amplifier (HrpRS) , finally is the output module made up by an inducible pHrpL promoter that receives the amplified signal from the cascade and promotes the expression of the reporter gene mRFP-Violet.

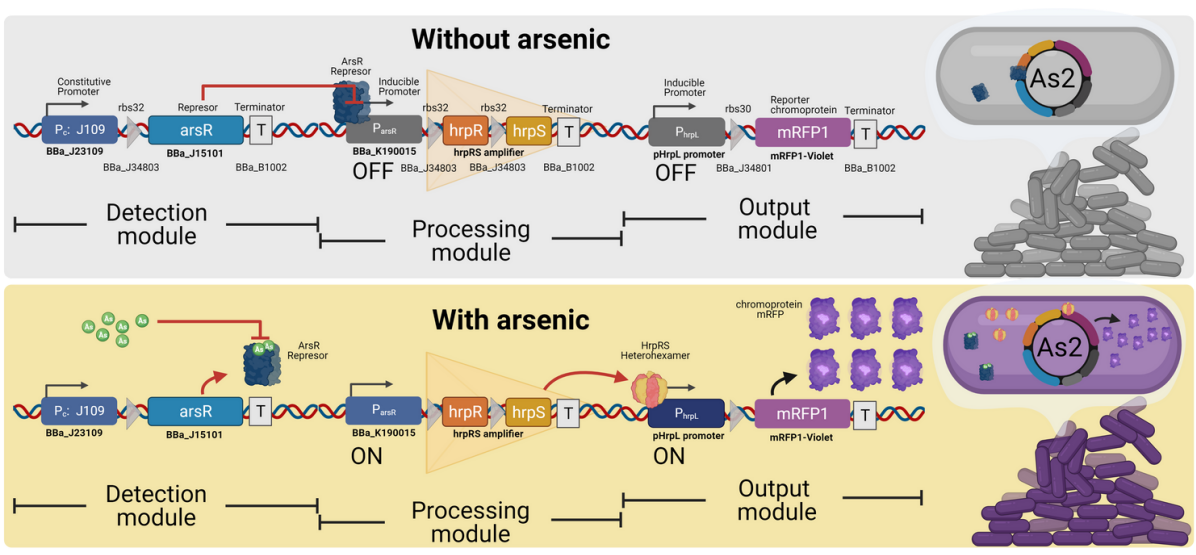


Figure 6.

3. As4 Construct (≥ 10 ppb [As] Sensitivity)

The detection module consists of a constitutive promoter of medium strength (BBa_J23115) that is in charge of the expression of the arsR protein (BBa_J15101) , in this construct a processing module is not included and immediately afterwards is the output module made up by Pars inducible promoter (BBa_K190015) that receives the signal from the detection module and promotes the expression of the reporter gene mRFP1-Violet.

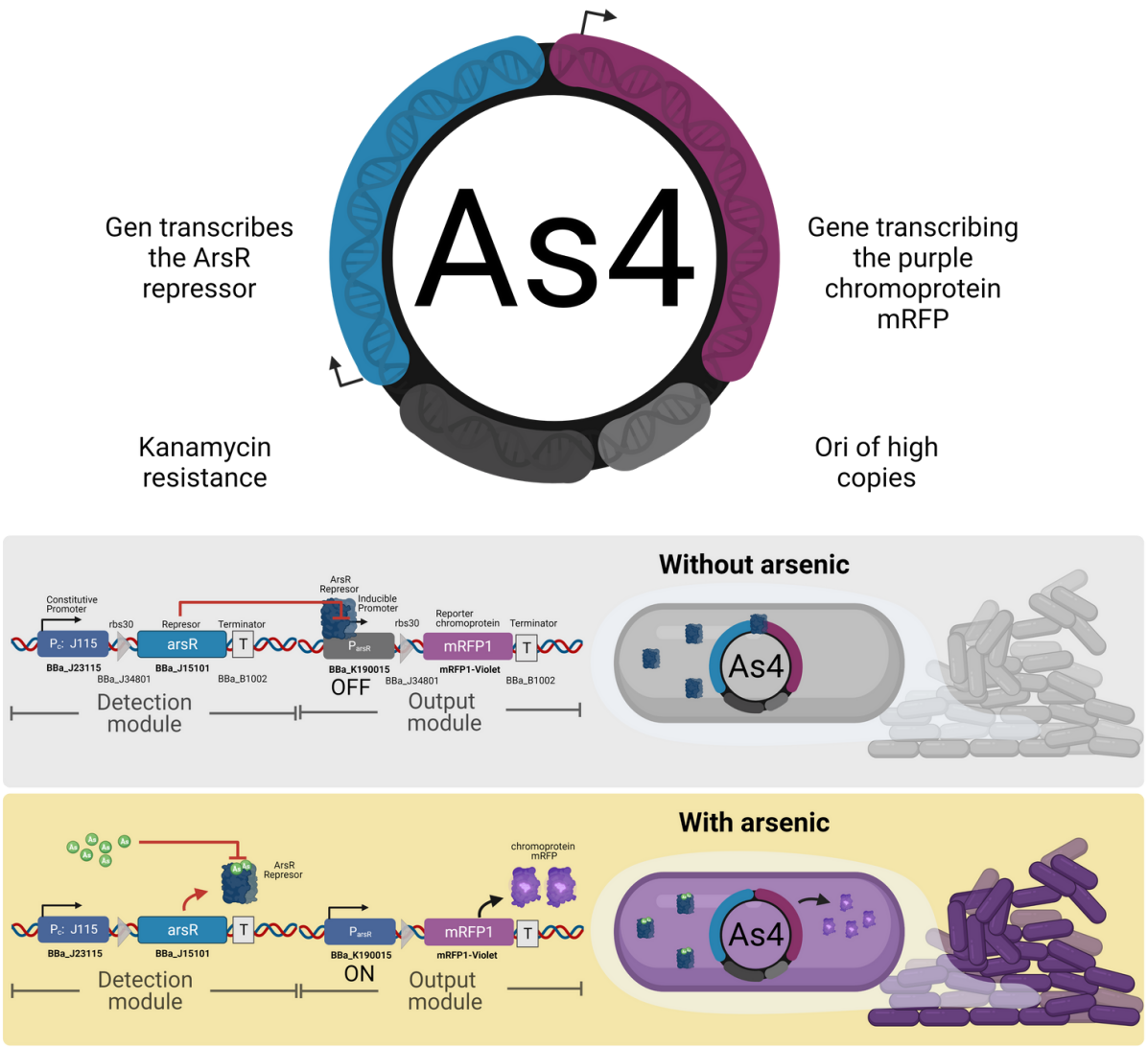


Figure 7.

4. As5 Construct (≥ 50 ppb [As] Sensitivity)

The detection module consists of a high-strength constitutive promoter (BBa_J23105) that is in charge of the expression of the arsR protein (BBa_J15101) , a processing module is not included in this construct and immediately afterwards is the output module made up by the inducible promoter Pars (BBa_K190015) that receives the signal from the detection module and finally promotes the expression of the reporter gene mRFP1-Violet.

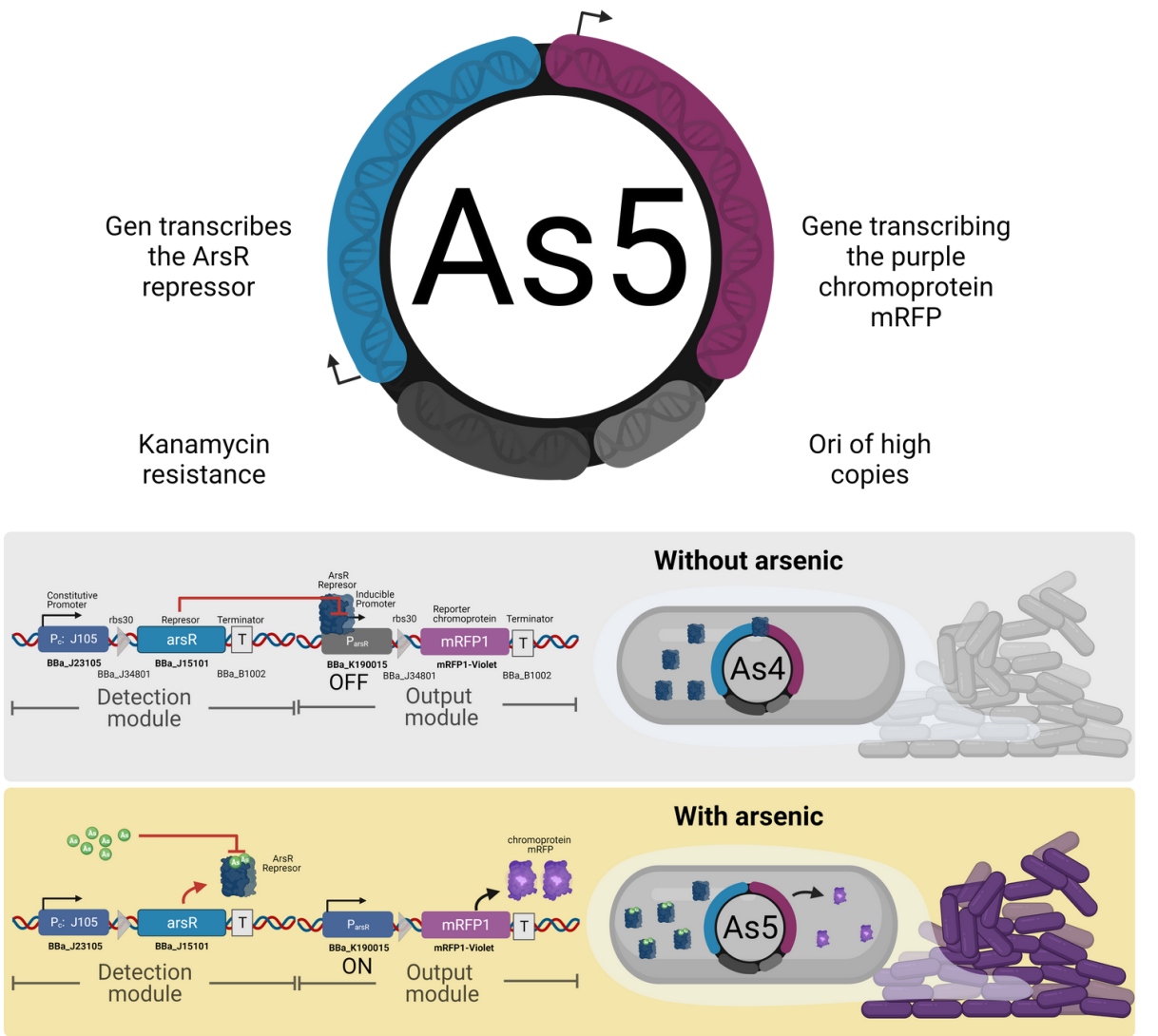


Figure 8.

5. Other considerations:

- Strong RBS sequences (BBa_J34801 and BBa_J34803) are located throughout the constructs coding genes.
- Terminator sequences (BBa_B1002) are positioned at the end of each coding gene.
- All constructs are inserted into pIDTSMART-Kan high-copy plasmids.

References

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