The Universal Tool For Gene Silencing

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The Idea

Small regulatory RNAs have untapped possibilities for synthetic biology applications and represent an active area of research. Such applications include convenient gene silencing and fine-tuning of gene expression, which are currently cumbersome processes restricted to well studied bacteria. We have investigated a novel type of RNA regulation based on the chitobiose system, where the inhibition caused by a small RNA is relieved by another small RNA called trap-RNA. We explore the possibility of using the system to uniquely target and repress any gene of interest, potentially providing unprecedented specificity and control of gene silencing.

Advantages

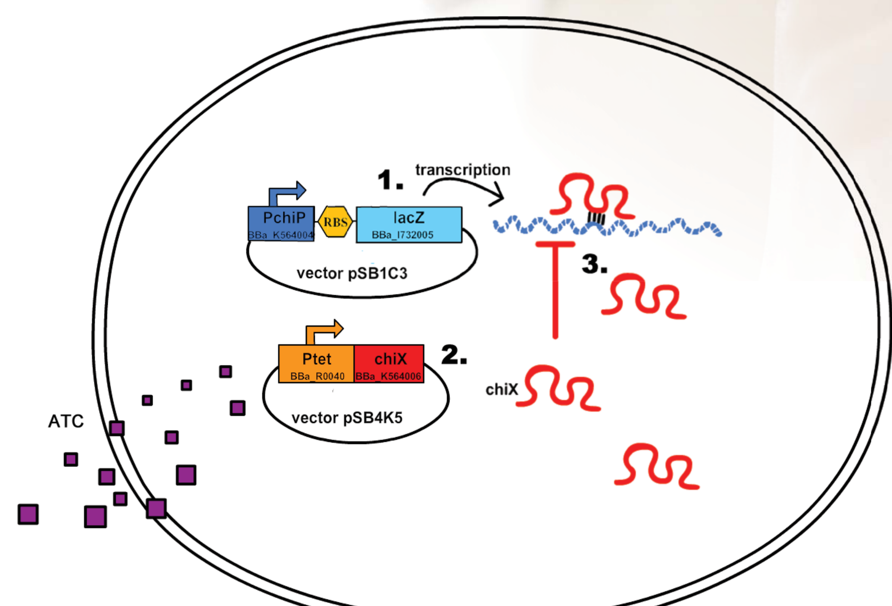
Our tool may has the potential to:

- Be highly modular
- Target many genes at once
- Enabling additional layer of regulation for increased control and tightness
- Potentially silence a single gene in an operon
- Keeping the genome wild type

The tool can function in most bacteria, it only requires the organism to:

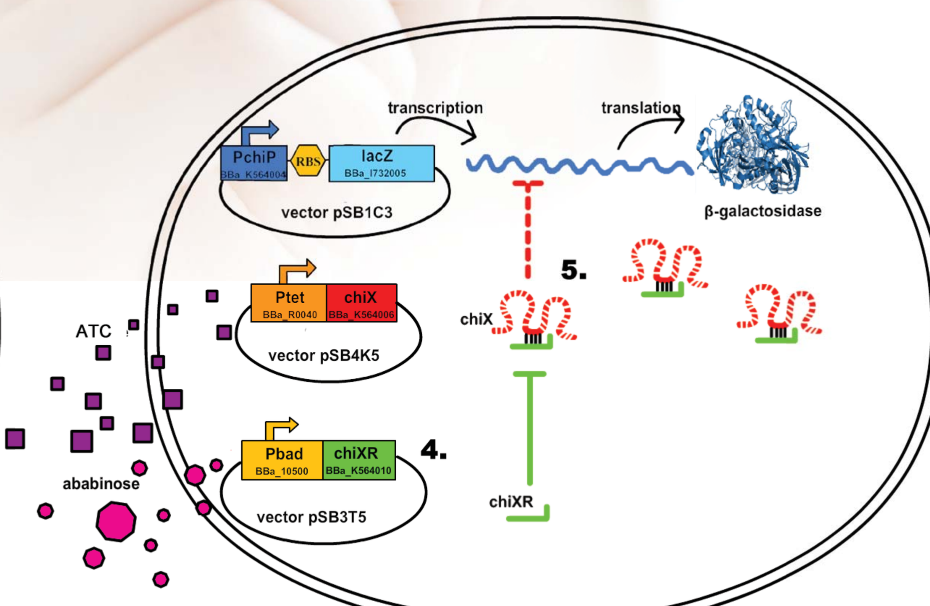
- Have sequenced RBS
- Contain Hfq
- Be able to receive plasmids
- Have known stable plasmids

Turning the gene OFF



1. The constitutive promoter and Ribosome Binding Site (RBS) for a chitoporin, (Salmonella chiP, E.coli ybfM), is fused with lacZ. In the absence of any regulation the cells produce lacZ.
2. A trans encoded sRNA (Salmonella chiX, E.coli micM, formerly rybC/sroB) is expressed under control of the inducible promoter P_{tet}.
3. The chiX sRNA triggers degradation of the chiP transcript by binding the RBS, lacZ mRNA is degraded, and lacZ is not produced.

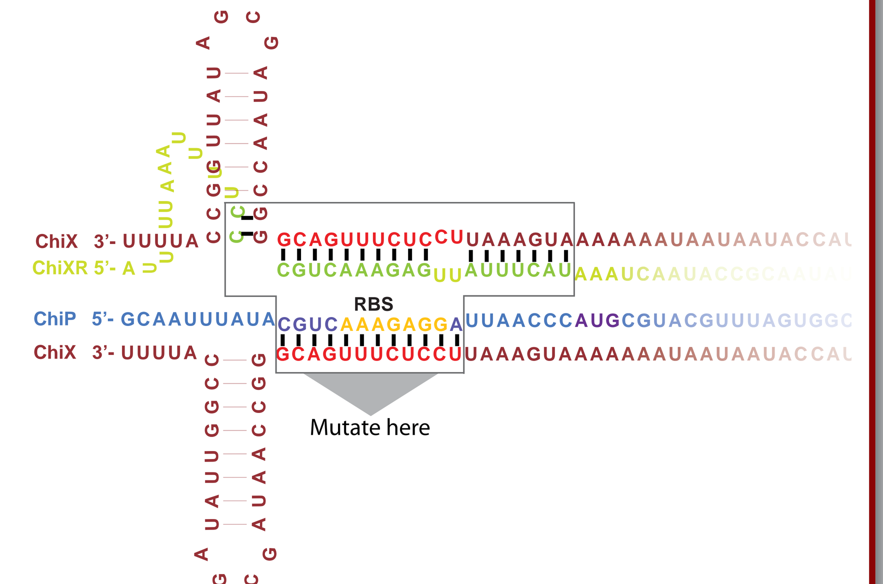
Turning the gene ON again



4. A trap-sRNA (chiXR for "chiX regulator") from an intergenic region in the chitobiose operon (chbBCARG) is expressed under control of P_{BAD}.
5. The trap-RNA relieves the LacZ gene silencing by degrading ChiX sRNA.

This is a proof-of-concept project, in theory, LacZ can be any gene.

Base pairing enables modularity



By simply mutating ChiX (red) and ChiXR (green) to match the ribosome binding site-region, any gene can potentially be targeted for regulation. The taget gene, (here ChiP) is shown in blue.

Examples of use

Knocking down essential genes: Using chromosomal engineering and repressible promoters, conditional knockout can be achieved, but the strains are no longer wild type. With our system, conditional and controllable silencing of any gene can be achieved.

Increase productivity in industrial bioproduction: Bacteria are often grown without expression and then induced to increase productivity. By being complementary to existing regulation, our system can silence leaky promoters.

Trouble-shooting: Rational design of biological circuits is often difficult and the right expression level of each component is almost impossible to predict. Using the proposed system, it could be possible to trouble-shoot designs of synthetic biology by quickly screening levels of gene expression.

Measuring and predicting sRNA silencing

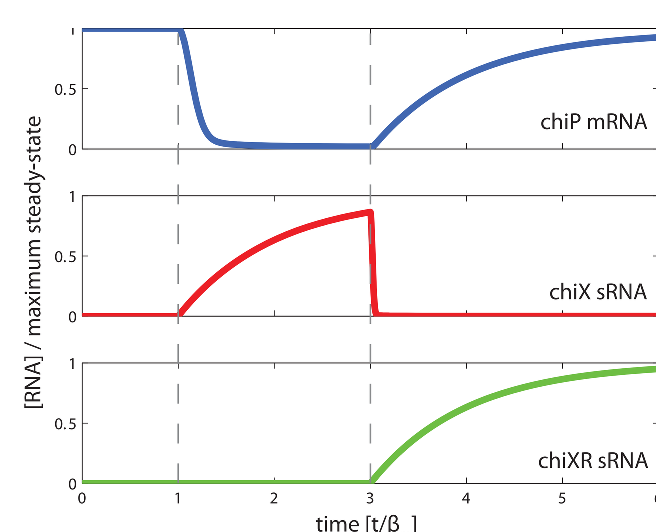
Engineering pairing region is sufficient

We measured the beta-galactosidase activity for two chiP-lacZ plasmids, one wild type and one with a mutated RBS. Two strains were used; one with chiX (NM522) and one without (IG302). It is clearly seen that chiX represses expression of LacZ in the plasmid with wild type chiP RBS but not the mutated one.

	Strain NM522 wt chiX	Strain IG302 Δ chiX	Fold Induction
ChiP WT RBS 5'- CGUCAAAGAGGA 3'- GCAGUUUCUCCU	467 ± 183	4781 ± 802	10,2
ChiP mutated RBS 5'- CGUCAAGGAGAA 3'- GCAGUUUCUCCU	2353 ± 314	2403 ± 297	1,02

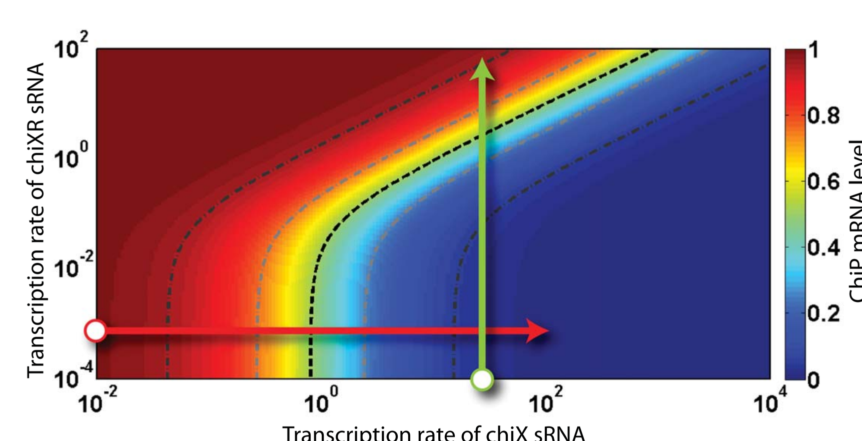
Beta-galactosidase activity. n=3.

Genes can be turned off and on



Dynamic simulation of the trap-RNA system. The two lines indicate induction events of the chiX and chiXR sRNAs respectively.

Genes can be fine-tuned to any level



A heatmap of the normalised steady state mRNA level. Red and green arrows are the direction of dynamic range when changing induction of sRNA and trap-RNA respectively. The axes are transcription rates of the sRNA and the trap-RNA.

In Short

Gene expression can be controlled in a dynamic fashion. Unlike inducible promoters, our system only requires a transformation, the strain is wild type until induction, and expression can be turned both on and off.

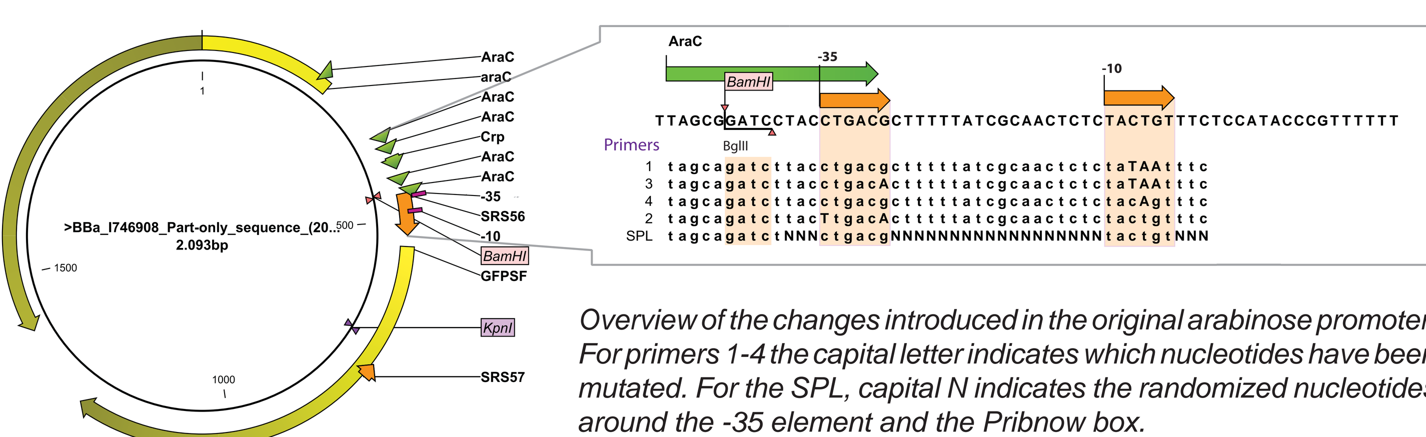
It is possible to fine-tune the final level of mRNA by controlling the level of the two small RNAs. This can be useful for lowering expression from a promoter that is too strong, or for reducing an unwanted gene product when doing metabolic engineering.

The first inducible Synthetic Promoter Library

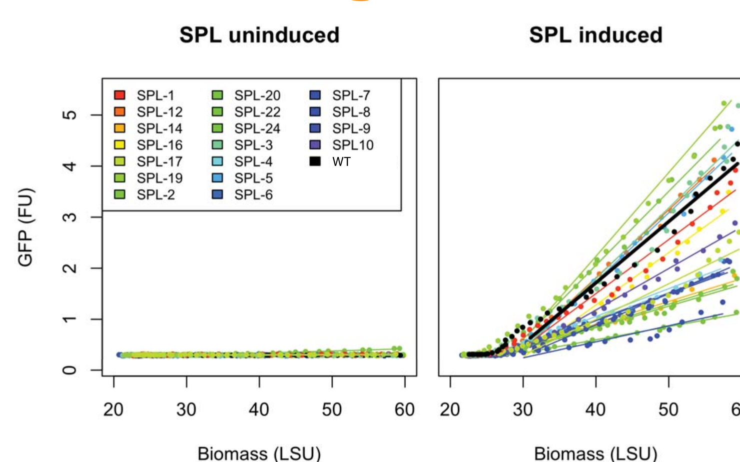
P_{BAD} is frequently used because it is tightly regulated. But it has several pitfalls:

1. *It is weak upon full induction.*
2. *It has an ON/OFF behavior making it difficult to tune.*

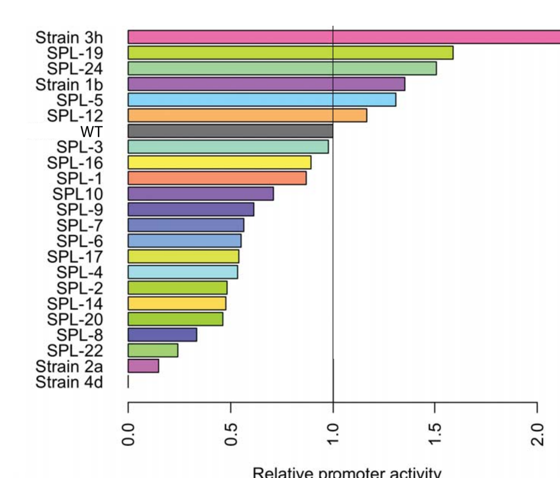
Since fine-tuning is essential in our project we approached these problems by making an inducible SPL with variable promoter activities, enabling the use of saturating amount of inducer, inspired by last year's DTU iGEM team's SPL.



Overview of the changes introduced in the original arabinose promoter. For primers 1-4 the capital letter indicates which nucleotides have been mutated. For the SPL, capital N indicates the randomized nucleotides around the -35 element and the Pribnow box.



Characterization of the P_{BAD} SPL. At the uninduced state (left) the expression of all promoters is similar to the original promoter (wt). In the induced state (right) the levels of GFP expression varies from 25% to 150% of the original promoter. This very small subset of tested isolates spans a wide range of promoter activities while still retaining the tightness of the arabinose promoter.



Comparison of the relative poromoter activity of both the rationally designed promoters (strain, 1b, 2a, 3h, and 4d) and the SPL. The SPL offers a range of promoter activities that once subcloned in front of chiXR would enable precise control of production.

Changing the arabinose promoter both rationally and randomly proved to be successful in terms of improving the dynamic range of this promoter at the saturation level of inducer. This approach extends the last year DTU team's efforts to improve constitutive promoters. It provides the framework for selecting an inducible promoter of a desired strength in a high-throughput manner. We believe that a similar approach can easily be applied to other inducible promoters, including the P_{tet}, to control production of chiX.

iGEM
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