

AND-gate DNA Circuit for Peptide Drug Secretion in E. Coli

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Background

While the development of treatment for colorectal cancer has happened around chemotherapy and radiotherapy, synthetic biologists have instead dedicated their work to the use of nonpathogenic bacteria such as *Salmonella typhimurium* and *Escherichia coli* [1]. Their natural preference for the hypoxia environment coincides with the cancerous environment found *in vivo*, and such a characteristic has been exploited by DNA circuit, quorum sensing, protein secretion, periodic lysis, etc [1]. Researchers have identified peptide drugs that have shown tumor regression either as mono-therapy or combined-therapy [2, 5, 3]. Thus, we propose an engineered *E. Coli* K-12 that is capable of the Peptide R production as a living treatment. Instead of the periodic global delivery via injection, the pro-biotic bacteria can be delivered via oral administration. Ideally, the introduced population will join the local micro-biome and localize to its favorable hypoxia environment near the tumor site and then the production of the peptide drug will be triggered by our DNA circuit.

Motivation

To deliver the drug to the tumor cells we want to design a circuit that produces the drug only in the conditions of a tumor. Hypoxia-inducible promoter, nirB, is naturally found in *E. Coli* [4], which provides a starting point for the engineering. However, the promoter is also activated by nitrite and nitrate thus limiting its ability as the sole signal for activation [4]. To reduce the false positive case, our engineered *E. Coli* K-12 will secrete peptide drug via a DNA circuit that is activated by hypoxia environment and quorum sensing. Once a population density is achieved, the engineered quorum sensing circuit will trigger the production peptide drug and thus maximizing pressure on tumor while minimizing the damage on healthy tissue.

Solution

We will engineer the *E. Coli* K-12 strain to secrete a therapeutic peptide via a DNA circuit that is only active in a hypoxia environment and when the density of bacteria is high. This will be implemented by combining a hypoxia induced promoter with a repressor that is repressed by quorum sensing to drive the synthesis of a secretion tagged peptide.

Approach

Design

Our design will include two plasmids, shown in Figure 1a. The first plasmid will consist of five inserts: (1) The LuxI-LuxR cassette with LuxR preceded by a high basal expression promoter and LuxI expression controlled by the Lux Box that the LuxR and AHL complex activate ([LuxI-LuxR](#)), (2) a coding region for an sgRNA (gRNA1) and will be activated by the same Lux Box, (3) a coding region for a second distinct sgRNA (gRNA2) that will have a promoter with high basal expression and will be repressed by gRNA1 when complexed with dCas9, (4) the peptide drug ([Peptide R](#)) and secretion tag ([Secretion tag](#)) coding region that is preceded by a hypoxia induce promoter, nirB ([nirB](#)), which will be repressed by gRNA2, (5) the coding sequence for GFP that will be expressed along with the peptide drug to enable testing. The second plasmid will contain the machinery for expressing the dCas9 molecules required for this CRISPRi system and we will insert a high basal expression promoter to ensure there is always dCas9 present in the system. When testing this circuit, multiple different Anderson promoters ([Anderson promoters](#)) will be tested for high basal expression and multiple spacer RNA sequences that bind to slightly different regions of the promoters will be tested. Every other part will be kept constant. The sequences for these plasmids can be found on Benchling ([Benchling](#)).

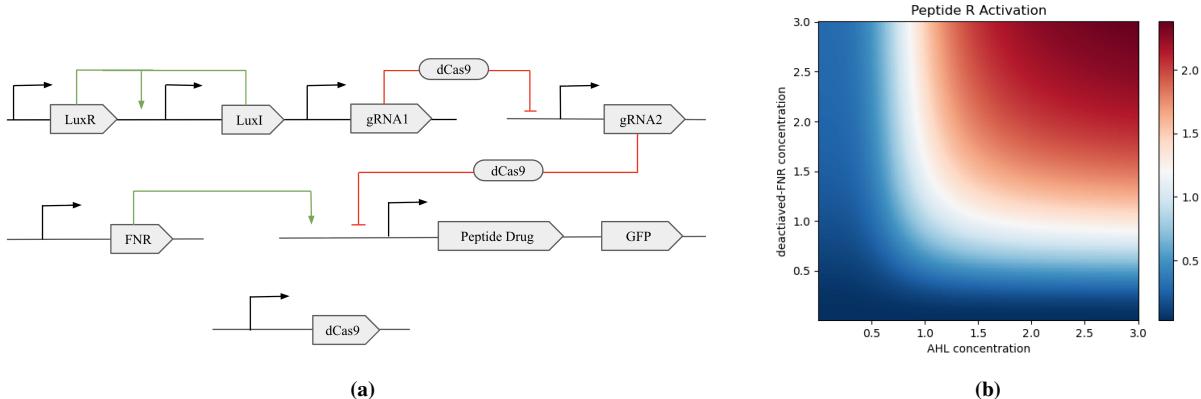


Figure 1: (a) The top right 3 components are implemented in one plasmid while the bottom dCas9 production is implemented in another plasmid. The FNR protein production is native to *E. Coli*. (b) The expected Peptide R production behavior under different environment.

Build

The smallest units of DNA that we will need are the primers for backbone amplification and the six inserts for the plasmids. It is most plausible to order all of these parts from IDT because in order to assemble the parts, we need to design overlaps on both ends of each insert. We will order the pBlade backbone ([pBlade](#)) as well as the pdCas9-bacteria backbone ([pdCas9-bacteria](#)) from Addgene. We will amplify both backbones using PCR and then use Gibson assembly to assemble the inserts into each backbone. Finally, the assembled plasmids are introduced to *E. Coli* K-12 and the culture will go through selection by the correct anti-biotic.

Test

To test the AND-gate logic in our design, we grow one group of the engineered *E. Coli* K-12 and extract the AHL from them while reserving a separate group for testing. Given that GFP and Peptide R shares the same *nirB* promoter, we assume the production of GFP coincides the production of Peptide R. Hence, we rely on the fluorescence intensity to test the steady state of Peptide R under different environment. And we need at least 4 test tubes: (1) the control group with high oxygen concentration and low AHL concentration; (2) low oxygen and low AHL; (3) high oxygen and high AHL; (4) low oxygen and high AHL. Within each tube, a small population of the engineered *E. Coli* is present to avoid naturally raised AHL level. A camera will be set up to capture fluorescent signal intensity from the tubes. The number of samples in each test could increase based on the need of data density.

Learn

The fluorescent intensity from the first testing stage will be computationally converted to the GFP concentration, which approximates the Peptide R concentration. Since the testing is done by tuning oxygen concentration, we assume the deactivated-FNR concentration is inversely proportional to oxygen. The data will be plotted to a heat map and compared against Figure 1b. The visualization will tell if the DNA circuit behaves like the AND-gate. Based on the difference of Peptide R concentration in FNR direction and AHL direction, we learn how switch-like the corresponding promoters are. Thus, we know what components of our DNA circuit to alter in the next DBTL cycle. The cycles can continue until we either run out of well-studied components, or we find one combination of hypoxia and quorum sensing promoter returns optimistic data.

Budget

Component	Amount × Unit Price = Total (Itemize different parts)	Justification
DNA	<p>2 plasmids × \$89 = \$178</p> <p>4 primers: $24b \times \\$0.45 = \\10.80 $26b \times \\$0.45 = \\11.70 $27b \times \\$0.45 = \\12.15 $22b \times \\$0.45 = \\9.90</p> <p>5 gBlocks: $1888\text{bp} \rightarrow \\198.50 $281\text{bp} \rightarrow \\50 $257\text{bp} \rightarrow \\50 $651\text{bp} \rightarrow \\72 $783\text{bp} \rightarrow \\95</p> <p>dCas9 insert: $84b \times 2 \times \\$0.84 = \\141.12</p>	The price for the plasmids was found on Addgene. The 4 primers will be ordered as oligos from IDT. The price for 25 nmole DNA Oligo is \$0.45 per base. The 5 inserts for the first plasmid will be ordered as gBlocks on IDT. The price for the gBlocks differs for different lengths of DNA, so the price for each separate insert is provided. The insert for the promoter for the dCas9 plasmid will be ordered as 2 DNA Oligos that will be annealed together. The price for 100 nmole DNA Oligo is \$0.84 per base.
Other resources	E. Coli strain K-12 → \$86	The price for E. Coli was found on ATCC (ATCC).
Total	\$915.17	We assume the equipment needed for fluorescent signal collection is provided and the critical biological components are all we need to order.

References

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