Milestone 5:

Combating Lactose Intolerance with a β-Gal and LDH Production Genetic Circuit

IBEHS 2P03

Riley Dowd

Huzafa Hyde

Nabil Johny

Veronica Marrocco

Ben Motz

Group 24

Submitted: April 11, 2022

Table of Contents

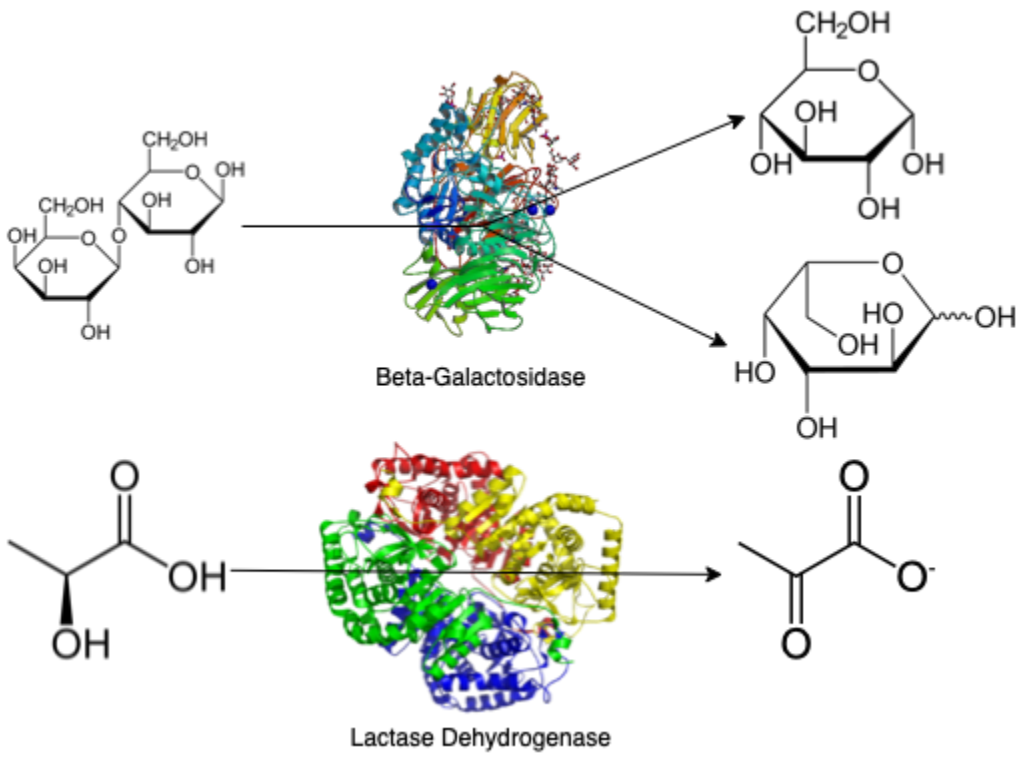


Figure 0: Illustration of natural lactose metabolism by Beta-Galactosidase and LDH, the desired products of the design genetic circuit.

Abstract

Lactose intolerance (LI) is defined as the failure to metabolize lactose into its monosaccharide components [1]. LI occurs primarily when the activity of lactase in the intestine is reduced or absent, allowing unabsorbed lactose to reach the colon where it is salvaged via bacterial fermentation [2][4]. Afflicting nearly 70% of the world's population, unpleasant LI symptoms include diarrhea and abdominal pain [3]. The byproducts of colonic lactose fermentation, particularly lactic acid, lower the gut lumen pH and further contribute to symptom progression [4].

Novel breakthroughs and advancement in the fields of synthetic biology and genetic engineering can be implemented to design high-precision, low-cost LI treatment solutions. This report highlights the applicability and efficacy of a genetic circuit, designed and simulated in SimBiology, to artificially upregulate β-Galactosidase (β-Gal) and lactose dehydrogenase (LDH) when lactose is malabsorbed. Simulations performed in SimBiology showcase the circuit's ability to digest lactose, produce β-Gal/LDH, and recover the gut pH.

Introduction

Lactose, the predominant carbohydrate in milk, is a disaccharide consisting of D-galactose bound to D-glucose. It is normally absorbed through the microvilli of the small intestines, first hydrolyzed into its monomer components via the enzyme lactase-phlorizin hydrolase, commonly referred to as lactase [1]. Glucose and galactose can subsequently be absorbed into the bloodstream and serve their various metabolic functions in the body [2].

Lactose intolerance (LI) is defined as the GI tract's failure or ineptitude to metabolize lactose into its monosaccharide components and occurs primarily when the activity of intestinal lactase is reduced or absent [1] [3]. Indeed, inadequate lactase activity allows lactose to reach the colon where it is metabolized into short chain fatty acids via bacterial fermentation. Byproducts of this decomposition include hydrogen, carbon dioxide and methane, causing flatulence in the colon and associated abdominal pain, two predominant symptoms of LI [4]. Moreover, unabsorbed lactose reaching the colon has a high osmotic load, leading to increased water in luminal contents, speeding transit and softening stool [5]. Overall, LI patients typically present with a number of gastrointestinal symptoms, including abdominal distension, cramps, flatulence and diarrhea following lactose consumption [6].

Current treatment of LI consists primarily of avoiding lactose-containing foods. Yet, a source of dairy provides key nutrients such as calcium, vitamins A and D, riboflavin and phosphorus; its restriction thus posing an increased risk for the development of nutritional deficiencies and a wide range of associated diseases [7]. Indeed, studies have established a comprehensive list of LI comorbidities, not only including osteoporosis and osteomalacia, which arise (evidently) from calcium deficiency, but also seemingly unrelated systemic problems such as cancer, neurodegeneration and diabetes [7] [8]. This vast clinical picture stresses the often overlooked public health significance of LI, as well as its sizable socio-economic burden.

Recent evidence further indicates that dietary lactose enhances calcium absorption and, conversely, that lactose-free diets result in lower calcium absorption. Thus, even a lactose-free diet that retains dairy may predispose individuals to inadequate bone mineralization, osteoporosis and osteomalacia [9]. This stresses the importance of exploring novel treatment solutions that effectively address the underlying mechanisms of LI *without* eliminating an entire food group. Unfortunately, the simplicity of LI as a disease and its "straightforward" treatment plan (that merely avoids symptoms rather than addressing the problem at hand) undermine its significance and conceal this need for novel treatment options. Indeed, LI gains very little attention from the media, is largely ignored by the scientific community and receives limited government funding [10].

Synthetic biology is a well-established scientific field that offers solutions to a span of societal problems. To manipulate the basic constituents of life is to manipulate life itself and redesigning physiologic systems can help tackle important healthcare challenges. In fact, genetically engineered bacteria have already proven effective in the treatment of infectious disease and cancer diagnostics [11]. As these breakthrough technologies continue to offer promising results, it is time to revisit the possibility of curing those conditions, like LI, once thought "incurable". This article puts forth a genetic circuit, designed and simulated using SimBiology, that effectively digests lactose, artificially rectifying LI patients' inability to produce lactase. This project is in the hopes that lactose intolerant individuals may one day be presented with a cure to their condition rather than short term symptom relief.

The main objective of this circuit is to enhance the activity of intestinal β-Gal and L-lactate dehydrogenase (L-LDH), which are endogenously produced. β-Gal mimics lactase's ability to hydrolyze lactose, while L-LDH is able to convert lactic acid into pyruvate, hence counteracting a drop in pH. This circuit is designed to switch between two functionalities in response to environmental triggers; lactose presence and low pH. It is expected to detect intestinal lactose and first hydrolyze it via β-Gal upregulation. The low pH resulting from this degradation process (i.e. production of lactic acid) would subsequently be detected and rescued via L-LDH activity, assuming lactose is still present and thus creating an AND gate for L-LDH regulation.

Materials and Methods:

Proposed Methods and Techniques for Wet Lab Circuit Implementation:

The anticipated construction of this biological circuit will utilize the BioBrick Standard Assembly method outlined in lecture [12]. Techniques such as PCR and gel electrophoresis will also be used as controls and checks to ensure that the experiment is running correctly, according to the cited protocols [13], [14].

First of all, a PCR should be run on any parts arriving from iGEM that may require amplification, including the LDH coding region and the pSB1A3 plasmid backbone. Primers have been designed for both cases (See **Figures 2,3**).

BioBrick assembly involves the repeated digestion and ligation of a backbone plasmid with a number of other DNA molecule(s), continuously cutting and pasting new sequences, or "*biobricks*", into the backbone, slowly building it up (see **Figures 4,5)** [12]. In general, four different restriction enzymes are used to achieve a double digest [12]. For this plasmid (pSB1A3), the required enzymes are Eco-R1, Xba1, Spe1, and Pst1. In accordance with the location of these sites, Eco-R1 and Spe1 will be used to digest the plasmid and Xba1 and Pst1 will be used to digest the DNA insert in question.

After digestion, the DNA must undergo purification to eliminate any lasting proteins or other cellular debris that could hinder ligation later on. Once purified, ligation can occur and the insert will be added to the plasmid, reforming the circular loop. This will be repeated a few times to generate the final plasmid sequence.

Finally, the ligated plasmid must run through a PCR for amplification [13]. Then, it will be transformed into the bacterial cell and the subsequently new strain of bacterial colonies will be streaked onto a petri dish [15], [16].

Controls and Checks:

In order to properly monitor and check the plasmid's assembly status, a gel electrophoresis should be performed on the plasmid after each part is inserted. In addition, to ensure the restriction enzymes effectively digested individual parts, they should also undergo gel electrophoresis following digestion [14]. Because the base pair length for each part should be acquired upon purchase, these experiments would serve to ensure that each part is properly digested and ligated into the plasmid. These steps should be implemented after purification to ensure it has been performed correctly.

Three different petri dishes should be prepared at the end. Each dish will contain some ampicillin, which the final prepared plasmid should be resistant to. The first will contain plated bacteria following every step correctly as outlined above. The second will contain bacteria that has not been transformed with the new plasmid. These bacteria should die when plated and therefore the plate will appear empty. This is to ensure that the regular bacteria have no resistance to ampicillin. The third will contain bacteria

that has been through the transformation step, however T4 DNA Ligase will not be added as it should have been in the ligation steps. The plasmid that should have been transformed is now just a bunch of strands of DNA that are floating around. This will not be able to transform into the bacteria, and therefore the plate should look empty as the ampicillin resistance is not present. This solution of multiple differing DNA fragments can be run through a PCR and a gel electrophoresis in order to visualize the lengths of DNA present [13], [14]. A gel electrophoresis can also be run on the final plasmids to test the length and ensure that proper ligation has occurred [14].

Experiments to Test Efficacy:

For the following experiments, 3 MacConkey agar plates will be used [20]. The purpose of these plates is to evaluate the circuit-carrying bacteria's aptitude in decomposing lactose. The plates should be designed according to a protocol published by *The American Society for Microbiology*, with gelatin, lactose monohydrate and peptones that provide key nutrients to facilitate the growth of bacterial colonies [20]. No glucose is made available in order to preserve lactose degradation as the sole metabolic pathway. Bile salts and crystal violet should also be added to selectively cultivate gram-negative bacteria, like *E. coli*, while preventing the growth of other species [20]. Finally, the MacConkey plate will contain a pH indicator, bromophenol blue, that turns yellow under acidic conditions [21]. While neutral red is the standard pH indicator used in the MacConkey plates, bromophenol blue is able to differentiate between low pH values, even lower than ~3 [22], meaning that lactic acid production (pH of ~2.43) would be detectable [23].

For testing the efficacy of our circuit, each MacConkey agar plate will be placed in a heating chamber set to 37°C, simulating the approximate temperature of the human gut and enabling bacteria to grow at a rapid rate [24]. Three separate tests will run on three separate plates (see **Figure 6**).

For the control reaction, wild type *E. coli* that did not undergo any genetic modification will be plated. This effectively simulates the bacterial fermentation of lactose, which occurs in the gut of patients with LI. Plated colonies would be expected to decompose the lactose monohydrate present for fuel and bacterial growth should be observed accordingly. It is additionally expected that the plate turns slightly yellow in color since bromophenol blue takes on a yellowish tint under acidic conditions. As *lacZ*-regulated β-Gal would gradually metabolize the lactose molecules, they would be converted to pyruvate, and then lactic acid, which ultimately creates an acidic extracellular environment [17] [18] [19]. If bacterial growth is limited and/or if the medium does not become yellow, this would be indicative of an underlying error in the experimental procedure, hence the utility of the positive control.

For the second plate, *E. coli* will be genetically modified to express the first portion of the biologic circuit designed above, which should enable lactose detection and subsequent β-Gal production. Mini-circuit insertion will follow the protocol presented in the study by Cheng *et al* [25]. Since this cultures' endogenous β-Gal expression is upregulated by the genetic insert, it is expected that their plate turns vibrant yellow as the overexpressed β-Gal continues to convert lactose into lactic acid. No additional, restorative color changes should be observed since the pH recovery feature has not been inserted into the *E. coli* strain at hand. This test solely ensures that the circuit-carrying bacteria can effectively hydrolyze lactose, whereas (assuming this test was successful) the following checks for the circuit's restorative pH abilities.

Indeed, the final MacConkey plate will host an *E. coli* strain carrying the full biologic circuit, which should theoretically allow for lactose detection along with the production of β-Gal and pH regulation, effectively counteracting LI *in vitro*. Circuit insertion will again follow the protocol presented

in the study by Cheng *et al* [25]. Therefore, if successful, the plated colonies should have an enhanced ability to hydrolyze the lactose monohydrate, as the circuit insert should successfully upregulate endogenous β-Gal activity. Several colonies should appear visible on the plate, similar in number to those observed on the two plates listed above. Also similar, the final plate should turn yellow due to the ultimate formation and expulsion of lactic acid. However, unlike in the other two plates, the low pH detector should subsequently activate and initiate the production of L-LDH. The plate should thus begin to turn to a lighter shade of yellow/green (potentially even back to the neutral blue color) as the pH slowly increases due to the conversion of lactic acid to pyruvate via L-LDH. Eventually, the color change should cease, indicating that the low pH detector is no longer being stimulated, and that baseline pH has been restored. This cycle should repeat with each new lactose molecule undergoing metabolism.

To aid in rapid error identification, chromoproteins will further be added to the circuit insert to track its progress in task completion. First, if the lactose detecting portion of the circuit is functional and β-gal is to be expressed, amilCP fluorescent proteins will be produced, tinting the cultures *blue* and thus providing a visual progress check. Similarly, the circuit will contain a cinI genetic sequence that initiates eforRed protein production upon successful low pH detection, which would tint the bacteria *red/purple* color and imply that L-LDH is to be produced.

Modeling Methods Using SimBiology Software:

The species displayed in the SimBiology model (**Figure 9**) are the same ones featured in the circuit. LacI, TetR, CinR, AmilCP, CinI, AmilGFP, CI, β-Galactosidase (β-Gal), lactate dehydrogenase (LDH), and the products of lactose metabolism and glycolysis are all included. The associated mRNAs of each species are also included, as well as their corresponding transcription, translation, and degradation rates. Certain pairs of particular species, i.e. LacI/lactose and CinR/CinI, form complexes within themselves and thus, their respective association, dissociation, and degradation rates are included as well. The values for each of these parameters are presented in Table 1 in the appendix.

In order to input equations into the Simbiology model, several assumptions were made regarding the species' behavior. For instance, it was estimated that the dissociation constant for the lacI/lactose complex was approximately equal to the inverse of the complex association constant. The base translation efficiency of all mRNA products (i.e. TetR, CinR, CinI) was also assumed to be 20 proteins/mRNA molecule as described in the coursepack. Furthermore, when parameter values were available online for select species but not all, the same value was applied to the remaining species. Such was the case when the affinity between lacI and the promoter DNA which it regulates was obtained (10^{-10} M [30]), but the affinity between H^+ ions and its Asr promoter (acidity detector) was not; the available value was applied to both species. Additionally, a dissociation constant was obtained for the lacI/lactose complex but not the CinI/CinR complex and thus, the same value was applied to both. Finally, when specific mRNA half-life, protein half-life, and degradation constant values for a species was unavailable, a blanket value was used, i.e. the constant for the corresponding component found endogenously in *E. coli* host cells [31] [32].

Numerous equations were incorporated into the model to generate accurate simulation graphs. Each mRNA species had a set of three associated equations; a rate of transcription, rate of translation, and rate of degradation. For instance, lacI mRNA had a rate of transcription solely based on the placIQ promoter [33]. The rate of degradation was simply the researched degradation constant multiplied by the concentration of lacI mRNA. Similarly, the rate of lacI translation was given by the mRNA translation constant multiplied by lacI mRNA concentration. Corresponding sets of equations were used in the

production of TetR, CinR, CinI, AmilCP, AmilGP, CI, β-Gal, and LDH. However, since the production of these species relies upon the presence of reactants that are not consistently present, their rates of transcription included terms that reflect the concentration of these conditional reactants. For instance, the production of TetR, CinR, and AmilCP are all inhibited by lacI and thus, their rates of transcription are described as the transcription rate constant multiplied by the inverse of lacI concentration over the lacI/DNA affinity, exponentially affected by their respective Hill constants (reference Table 2 for full list of equations). A similar phenomenon is observed in several other species including, CinI and AmilGFP which are both generated in the presence of H^+ ions and therefore production is directly proportional to $[H^+]$. CI production is based on [TetR], β-Gal production is reliant on [CI], LDH production is reliant on the concentration of the CinI/CinR complex, and lactose metabolism only occurs when β-Gal is present. All of these species feature similar transcription rate equations with their respective reactants.

Results and Discussion:

Circuit Function Under Simulation:

In **Figure 9**, all the reactions and species are evident in the circuit model produced in Simbiology. The circuit's activity relies primarily upon the presence of lactose, which, under simulation, initiates the production of several cascading species and complexes, such as LDH and the CinI/CinR complex. When lactose first enters the subject, it forms a complex with the lacI that is naturally occurring in the body. Under this complex state, lacI is no longer able to inhibit the production of TetR, CinR, nor AmilCP, which are all produced via the same PL8-UV5 promoter. Therefore, when lactose is bound, these proteins increase in concentration, causing a cascade of events that ultimately result in β-Gal and LDH expression.

First, the increased levels of TetR that arise from lacI-lactose binding inhibit the production of CinI, thereby enabling the production of β-Gal. In **Figure 9**, notice β-Gal mRNA is negatively regulated by CinI when it is present. In the lactose-binding case, upregulated β-Gal catalyzes the metabolism of lactose into glucose and galactose, monosaccharides which can subsequently undergo glycolysis. However, the products of said glycolysis, particularly lactic acid, which is heavy in H+, contribute to the generation of a low pH environment. This environment type positively regulates the ASR promoter, which in turn stimulates the production of CinI and AmilGFP. Thus, freshly produced CinI and CinR, which was previously upregulated by lacI-lactose binding, are able to bind and form the CinRI complex. Finally, this complex initiates the transcription of LDH, thereby allowing the foreign lactose to be enzymatically handled and digested whilst simultaneously maintaining physiological pH.

Lactose/lacI regulates TetR, cinR and amilCP:

In **Figure 10**, lactose is present at an initial concentration of 50 mM as shown by the steep vertical line. LacI successfully binds lactose, under which state its inhibitory capacities are lost. Thus, levels of CinR (*red*), TetR and amilCP (*purple*) rise as seen in fig. 10. However, since lactose metabolism occurs rapidly, these levels fall back to zero as the lacI-lactose complex dissociates and inhibition is restored.

***Note that CinR does not reach the same heights as its counterparts TetR and amilCP since it is rapidly lost in binding to CinI to form the appropriate complex.

Low pH (i.e. lactose is present) produces amilGFP and CinI:

In **Figures 11-12**, lactose is present at an initial concentration of 50 mM. This lactose is decomposed via β-Gal and under simulation, its constituents undergo glycolysis, creating an acidic environment. This is evidenced by the green pH curve, for which there is an initial drop due to lactose. Once this drop is detected, however, ASR is activated, causing amilGFP and CinI levels to increase over time, before reaching a stable equilibrium as seen in the yellow curve. CinI/CinR further activate LDH which restores pH, as evidenced by the green curve's rise and eventual equilibrium state.

CinI+CinR complex produces LDH:

In **Figures 13-14**, lactose is present at an initial concentration of 50 mM. The orange curve shows LDH concentration, which increases to some equilibrium as lactose is metabolized through the system. As seen in the yellow curve below, the CinRI complex additionally spikes as it's being formed, in turn producing increasing amounts of LDH. Eventually, the complex is depleted and levels return to zero.

TetR produces CI to produce beta-Gal:

In **Figures 15-16**, lactose is present at an initial concentration of 50 mM. This leads to upregulation of tetR expression, which acts to inhibit CI and ultimately, promote β-gal production as shown by the black curve. Zoomed in, the graph shows that since lacI is inhibited, tetR is at a high concentration, reaching a high of ~ [200] before the lactose is depleted and levels return to zero.

Conclusions

In this paper, a biologic circuit was designed with the ability of β-Gal upregulation and pH rescue. Engineered bacteria equipped with this circuit should be able to flexibly adapt to variations in the intestinal environment, thus timely digesting lactose and rescuing the intestinal pH drop. Therefore, using engineered bacteria equipped with this circuit has the potential to serve as a promising method for treatment of LI.

This circuit prototype has limitations. First, its functionality has not yet been verified *in vivo* and the proposed wet lab experiments must still be performed to assess biocompatibility. If these were indeed successful, to apply the circuit to humans would still require more sophisticated studies in order to determine a proper chassis and ensure safety. Biocompatibility within the human gut is indeed a major concern. The *E. coli* host must eventually be administered into the gut lumen to assess its impact on the native gut microbiota and its potential strain on resources. Antibiotics may be added to reduce its effects. In short, extensive testing, prototyping and retesting will be required *in vivo* on *E. coli*, mice, and eventually humans, before the therapy can be safely offered to patients. Altering human genetics is no light matter and ethical boards must ensure the benefit is worth the risk.

As stated, while lactose intolerance is a seemingly futile disease, merely an inconvenience to most, there remain multiple ethical factors to consider with this proposed synthetic biology design. Lactose intolerance is concentrated in developing countries [9], where access to healthcare is already limited and treating LI is certainly not the first priority. The problem thereby spans into politics and poverty, over which biologists and researchers have seldom control. Should a project solution be proposed to a low-income country, a corrupt government or a business giant, its honest and effective implementation becomes uncertain, perhaps even improbable and costs could be overwhelming.

Despite these limitations and ethical considerations, the biologic circuit highlighted in this paper can serve as a promising prototype for the relief and eventual elimination of lactose intolerance. Due to its ability to flexibly adapt to environmental variation, in particular to stabilize colon pH and maintain β-Gal

activity after lactose influx, the circuit presented here is unlike any currently available treatment. Indeed, it could revolutionize the field with an adamant *cure* for LI, as opposed to short term symptom relief. Likewise, using similar methodology, diseases and allergies such as gluten intolerance have the potential to be cured as well. In conclusion, such controversial technologies come down to a question of worth vs. risk: how desperately do individuals afflicted with LI seek alternate treatment solutions and what could their implementation cost the world?

Appendix

<table>
<tr><th colspan="4">Materials Needed</th></tr>
<tr><th colspan="2">Construction of Circuit</th><th colspan="2">Testing of Circuit</th></tr>
<tr><td colspan="2">Restriction Digest Enzymes</td><td colspan="2" rowspan="4">MacConkey Plating
● Gelatin
● Peptones
● Lactose monohydrate
● Bile salts
● Crystal violet
● Bromophenol blue
● 6 MacConkey plates</td></tr>
<tr><td colspan="2">● Eco-R1
● Xba1
● Spe1
● Pst1</td></tr>
<tr><td colspan="2">Purification</td></tr>
<tr><td colspan="2">● Isopropanol
● Ethanol</td></tr>
<tr><td colspan="2">Ligation</td><td colspan="2" rowspan="8"></td></tr>
<tr><td colspan="2">● T4 DNA Ligase</td></tr>
<tr><td colspan="2">Polymerase Chain Reaction</td></tr>
<tr><td colspan="2">● Taq Polymerase
● Primers</td></tr>
<tr><td colspan="2">Gel Electrophoresis</td></tr>
<tr><td colspan="2">● DNA ladder
● Gel
● Loading dye</td></tr>
<tr><td colspan="2">Transformation and Plating</td></tr>
<tr><td colspan="2">● LB-Media
● Ampicillin</td></tr>
</table>

Figure 1: Basic materials list. All materials above can be sourced from Thermo Fisher [26].

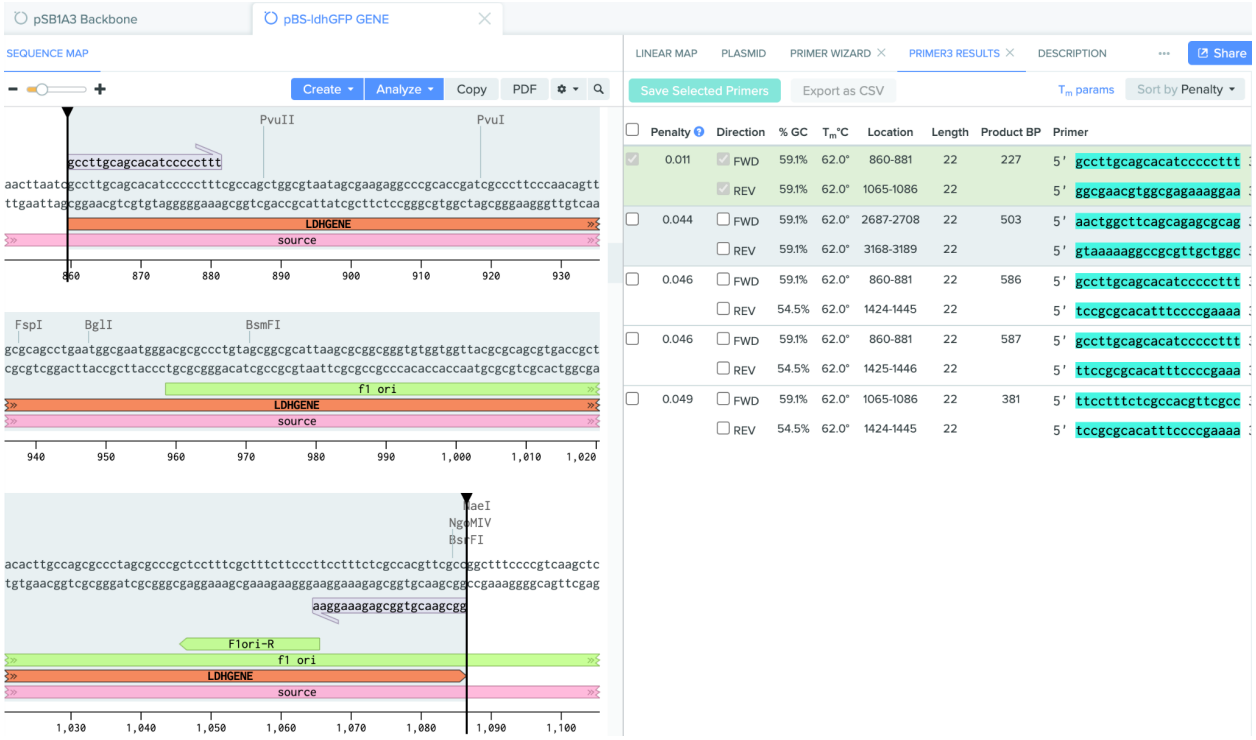


Figure 2: Primers located on the LDH gene insert for PCR amplification.

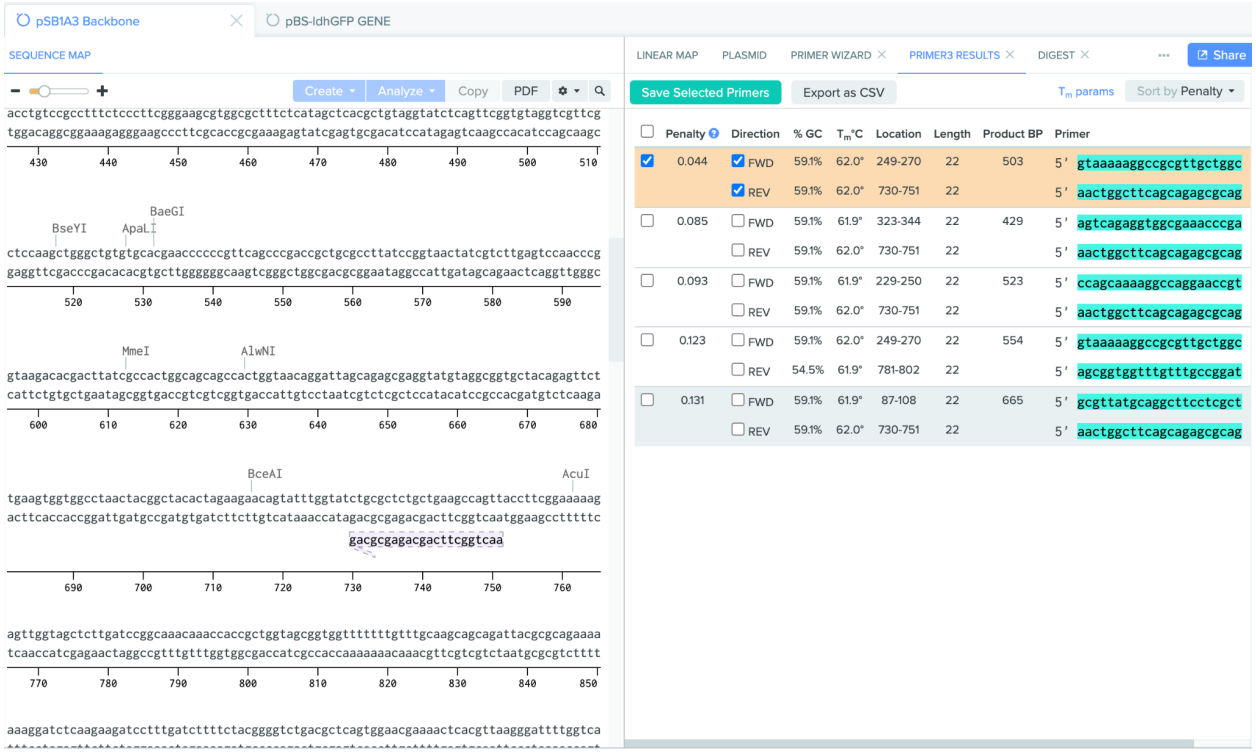


Figure 3: Primers located on the pSB1A3 backbone for PCR amplification.

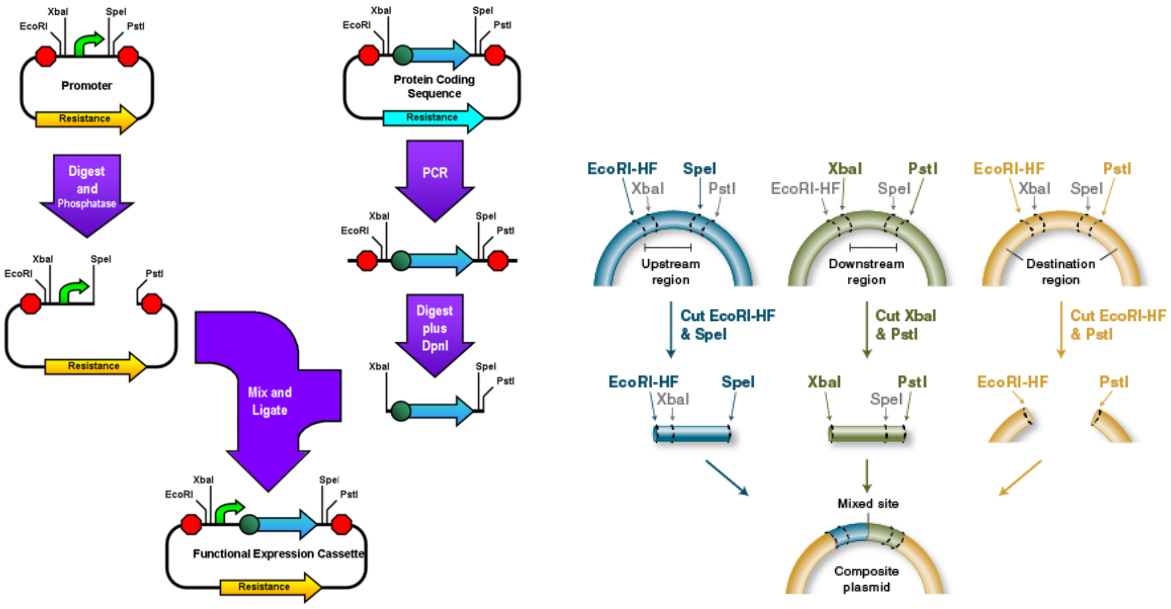


Figure 4 and 5: Visuals demonstrating the BioBrick assembly method [27].

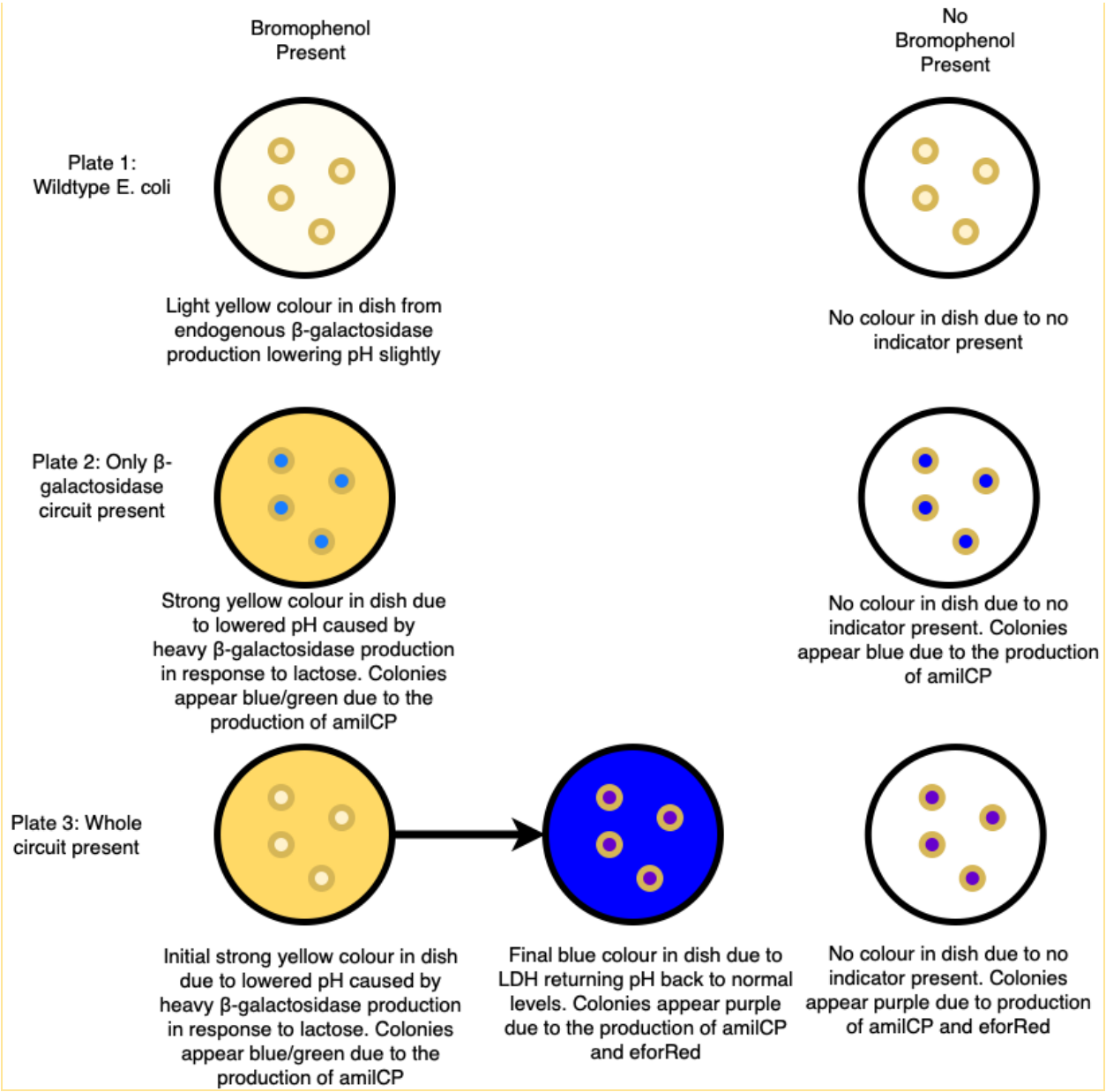


Figure 6: Summary of expected results of experiments to test efficiency.

Part	Function	Relation + Rational+Acquisition
BBa_J23055	*E. coli* chassis to carry completed plasmid	Fits with other parts as well as plasmid backbone
pSB1A3	pSB1A3 plasmid backbone	Contains proper restriction sites for BioBrick assembly, compatible with *E. coli*
BBa_J61100	Regular affinity RBS	Allows ribosomes to bind allowing for translation
BBa_B0010	Terminator (T1)	Typical terminator
BBa_K1695000	Lactose-inducible promoter	Detects high lactose levels (lactose would be operator)
BBa_C0040	Codes for a signaling protein (tetR)	Will be produced during high lactose levels along with cinR
BBa_C0077	Codes for a signalling protein (cinR)	Complex with cinI activates cin promoter, activated by high lactose levels along with tetR
BBa_K1231000	asr promoter with RBS, induced by low pH	Will be induced by low pH and activate L-LDH downstream
BBa_C0076	Codes for a signalling protein (cinI)	Complex with cinR activates cin promoter, activated by low pH
BBa_R0040	tetR repressible promoter (tetO)	Used in the NOT gate for β-galactosidase production
BBa_C0051	Codes for a signaling protein (CI)	Used in the NOT gate for β-galactosidase production
BBa_R0051	CI repressible promoter (CIO/pR)	Used in the NOT gate for β-galactosidase production
BBa_K2418000	Transforms lactic acid into pyruvate (LDH)	Recovers the PH in the gut. Pyruvate is then released for Krebs
BBa_I732005	Codes for β-galactosidase (lacZ)	Necessary to break down lactose into glucose and galactose, activated during high lactose levels
BBa_K592009	Codes for amilCP	Will be present in high lactose concentrations, ensures sensor is working by emitting a blue colour
BBa_K592012	Codes for eforRed	Will be present in low pH, ensures sensor is working by emitting a red colour

Figure 7: Updated part list including the chassis, backbone, RBS, terminator, and new signaling proteins. All parts can be sourced from iGEM [29].

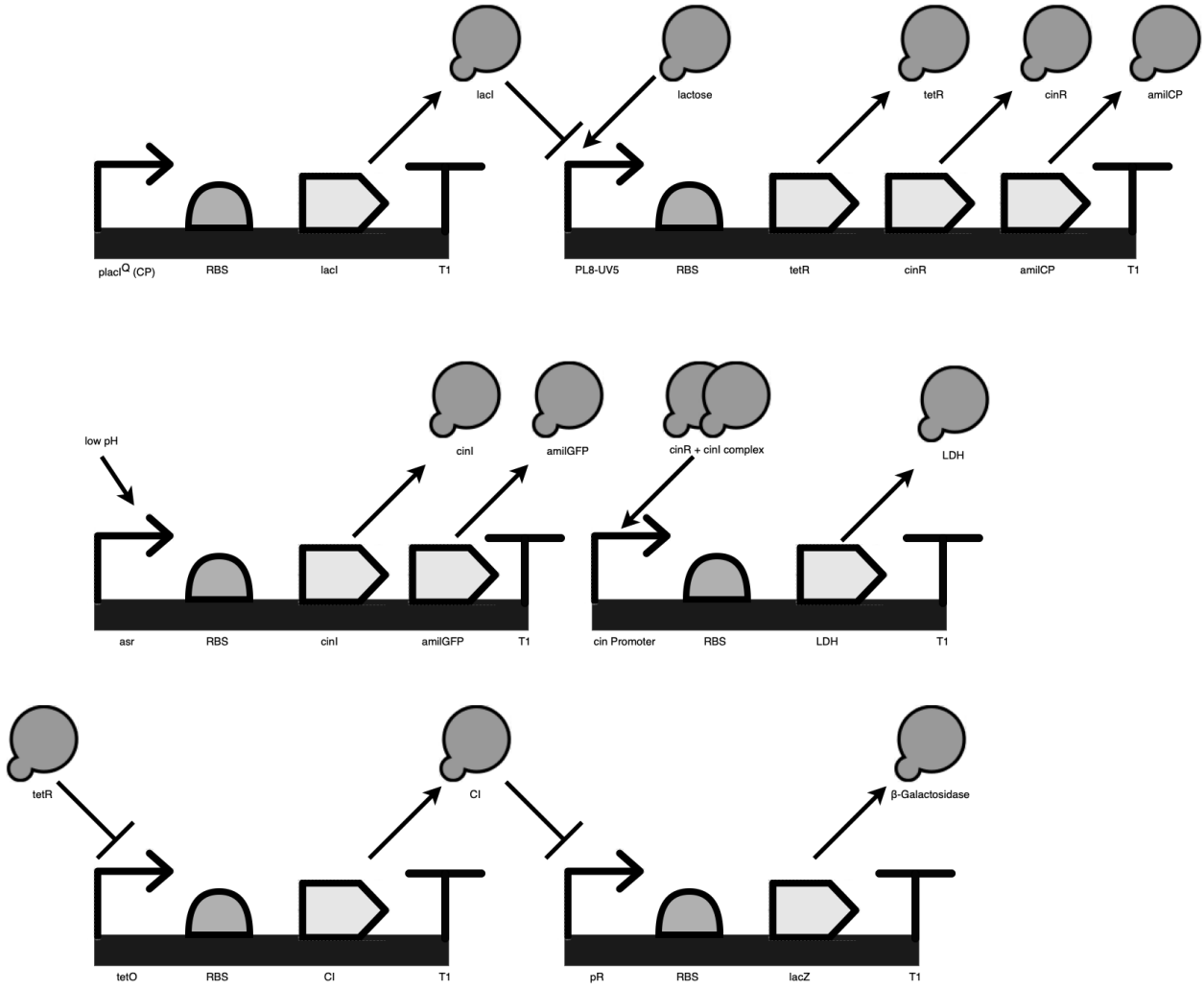


Figure 8: SBOL diagram outlining circuit promoters, species, RBS, reactions, and products.

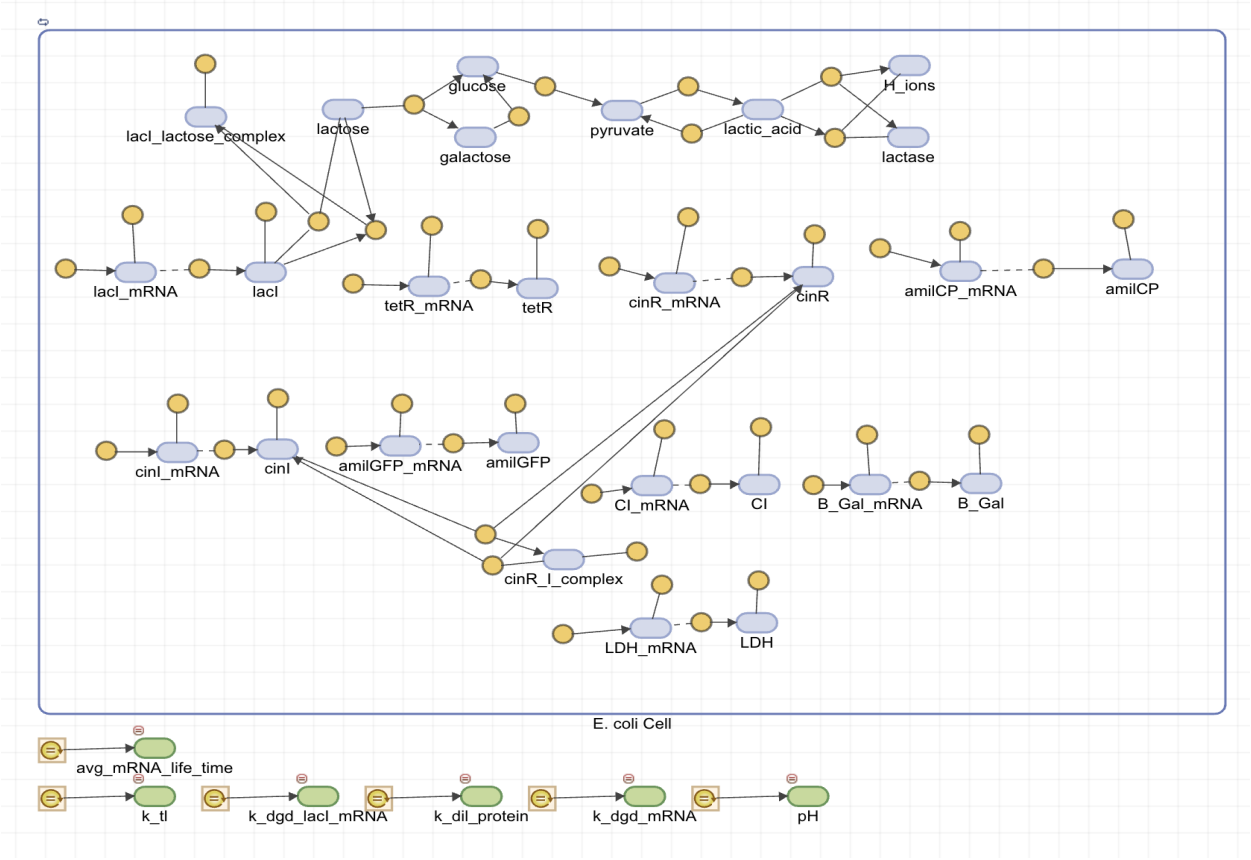


Figure 9: Circuit model produced in Simbiology.

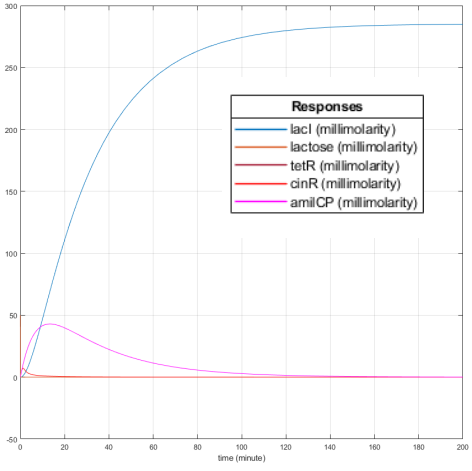
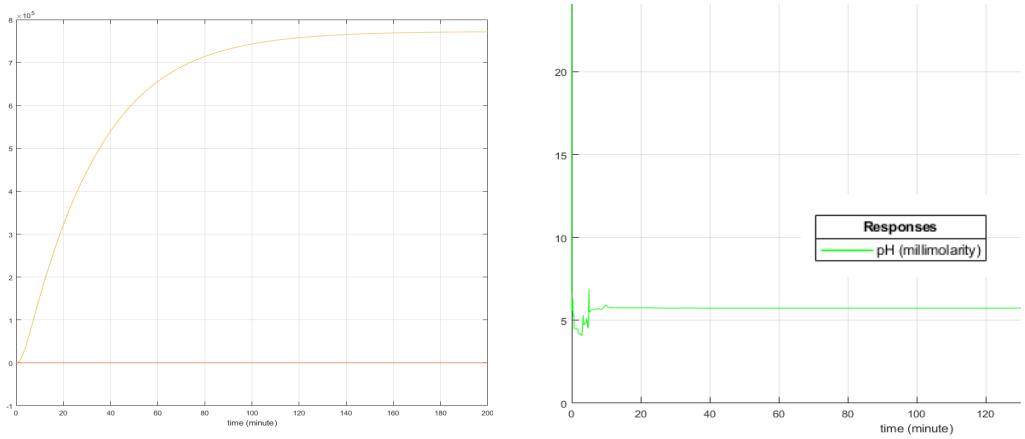
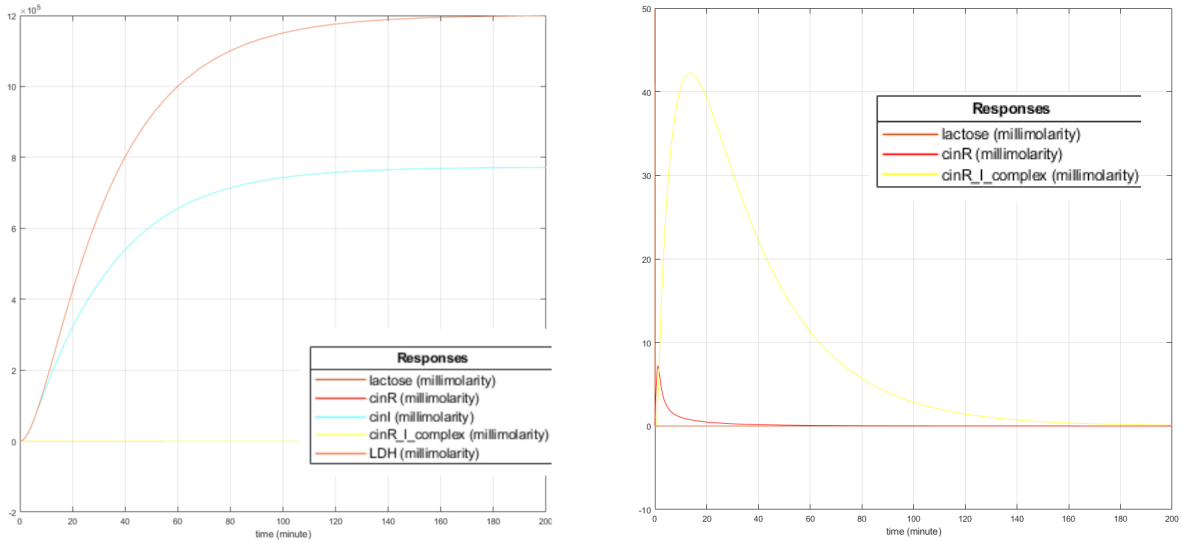


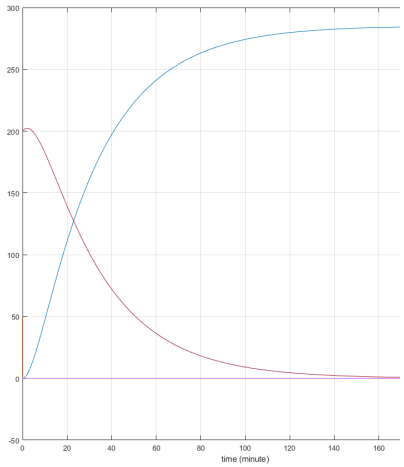
Figure 10: Lactose is present at an initial concentration of 50 mM.

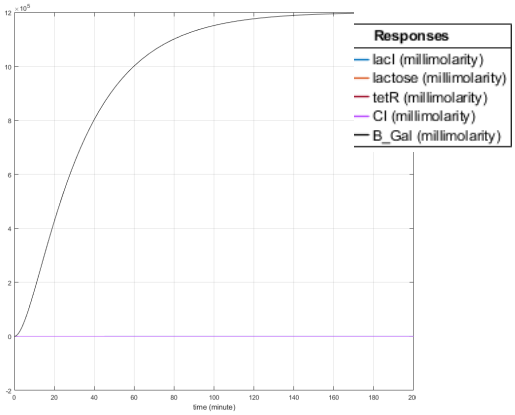


Figures 11-12: Lactose is present at an initial concentration of 50 mM.



Figures 13-14: Lactose is present at an initial concentration of 50 mM.





Figures 15-16: Lactose is present at an initial concentration of 50 mM.

Table 1: Constant Parameters Used in Simbiology Model

*Note that unreferenced values were taken from the course pack

Parameter	Value	Units	Description
k_CP_trsc	0.0125 [33]	mM/min	Constitutively promoted rate producing lacI_mRNA
Translation_ eff	20*	dimensionless	Ribosomal efficiency
avg_mRNA_ half_life	5*	min	Average mRNA half life for *E. coli*
lacI_mRNA_ half_life	3.8 [33]	min	lacI mRNA half life in *E. coli*
protein_half_ life	20 [31]	min	Average cell cycle time for protein dilution
k_lacI_ Complex_ assoc	0.5128 [34]	1/(M*min)	Association constant for the lacI-lactose complex
k_lacI_ Complex_ dissoc	1.95 [34]	1/min	Dissociation constant for the lacI-lactose complex
k_trsc	40*	mM/min	Transcription constant for all mRNA
K_lacI_DNA _affinity	$1 * 10^{-9}$ [30]	M	Affinity for lacI_promoter binding
K_tetR_ DNA_ affinity	$1.7857 * 10^{-10}$ [35]	M	Affinity for tetR_promoter binding
K_CI_DNA_ affinity	10 [36]	nM	Affinity for CI_promoter binding
hill_lacI	1 [37]	dimensionless	Hill coefficient for lacI_promoter binding
hill_tetR	2 [38]	dimensionless	Hill coefficient for tetR_promoter binding
hill_CI	2 [39]	dimensionless	Hill coefficient for CI_promoter binding

Parameter	Value	Units	Description
B_gal_kCat	750 [40]	1/s	β-Galactosidase catalyzation constant
B_gal_Km	0.12 [40]	mM	β-Galactosidase Km constant
galK_Vmax	191 [41]	μmol/min	Maximum reaction velocity for galactokinase
galK_Km	0.895 [41]	mM	Galactokinase Km constant
PFK_Vmax	194 [42]	μmol/min	Maximum reaction velocity for phosphofructokinase
PFK_Km	2.3 [42]	mM	Phosphofructokinase Km constant
LDH_kCat	245 [43]	1/s	Lactate Dehydrogenase catalyzation constant
LDH_Km	0.26 [44]	mM	Lactate Dehydrogenase Km constant
K_pyruvate_ ferm	0.0011 [45]	1/s	Pyruvate to lactic acid fermentation speed
k_acid	$1.4 * 10^{-4}$ [33]	1/s	Lactic acid k_a
k_base	$7.14 * 10^{-11}$ [33]	1/(M*s)	Lactic acid k_b

Table 2: Equations incorporated into Simbology model

Species	**Equation**
Average mRNA Lifetime (Universal Value)	log(2)/(average mRNA half-life)
Translation Constant (Universal Value)	(Translation Efficiency)/(average mRNA half-life)
mRNA Degradation Constant (Universal Value)	log(2)/(average mRNA half-life)
Protein Dilution/Degradation Constant (Universal Value)	log(2)/(protein half life)
pH (Universal Value)	-log([H^+])
lacI Degradation Constant	log(2)/(lacI mRNA half-life)

References

[1] D. H. Juers, B. W. Matthews, and R. E. Huber, “LacZ β-galactosidase: structure and function of an enzyme of historical and molecular biological importance: LacZβ-Galactosidase,” *Protein Sci.*, vol. 21, no. 12, pp. 1792–1807, 2012.

[2] A. Shaukat, “Systematic review: Effective management strategies for lactose intolerance,” *Ann. Intern. Med.*, vol. 152, no. 12, p. 797, 2010.

[3] R. Catanzaro, M. Sciuto, and F. Marotta, “Lactose intolerance—old and new knowledge on pathophysiological mechanisms, diagnosis, and treatment,” *SN Compr. Clin. Med.*, vol. 3, no. 2, pp. 499–509, 2021.

[4] T. He *et al.*, “Colonic fermentation may play a role in lactose intolerance in humans,” *J. Nutr.*, vol. 136, no. 1, pp. 58–63, 2006.

[5] N. L. Christopher and T. M. Bayless, “Role of the small bowel and colon in lactose-induced diarrhea,” *Gastroenterology*, vol. 60, no. 5, pp. 845–852, 1971.

[6] A. Farhana and S. L. Lappin, “Biochemistry, Lactate Dehydrogenase,” in *StatPearls [Internet]*, StatPearls Publishing, 2021.

[7] S. A. Abrams, I. J. Griffin, and P. M. Davila, “Calcium and zinc absorption from lactose-containing and lactose-free infant formulas,” *The American Journal of Clinical Nutrition*, vol. 76, no. 2, pp. 442–446, Aug. 2002, doi: 10.1093/ajcn/76.2.442.

[8] R. Schiffner, K. Kostev, and H. Gothe, “Do patients with lactose intolerance exhibit more frequent comorbidities than patients without lactose intolerance? An analysis of routine data from German medical practices,” *Ann. Gastroenterol.*, vol. 29, no. 2, pp. 174–179, 2016.

[9] R. Qiao, C. Huang, H. Du, G. Zeng, L. Li, and S. Ye, “Milk consumption and lactose intolerance in adults,” *Biomed. Environ. Sci.*, vol. 24, no. 5, pp. 512–517, 2011.

[10] W. J. Wooten and W. Price, “Consensus report of the National Medical Association. The role of dairy and dairy nutrients in the diet of African Americans,” *J. Natl. Med. Assoc.*, vol. 96, no. 12 Suppl, pp. 5S-31S, 2004.

[11] N. Saeidi *et al.*, “Engineering microbes to sense and eradicate Pseudomonas aeruginosa, a human pathogen,” *Mol. Syst. Biol.*, vol. 7, no. 1, p. 521, 2011.

[12] R. Shetty, M. Lizarazo, R. Rettberg, and T. F. Knight, “Chapter thirteen - Assembly of BioBrick Standard Biological Parts Using Three Antibiotic Assembly,” in Methods in Enzymology, vol. 498, C. Voigt, Ed. Academic Press, 2011, pp. 311–326. doi: 10.1016/B978-0-12-385120-8.00013-9.

[13] L. Garibyan and N. Avashia, “Research Techniques Made Simple: Polymerase Chain Reaction (PCR),” J. Invest. Dermatol., vol. 133, no. 3, p. e6, Mar. 2013, doi: 10.1038/jid.2013.1.

[14] M. A. Hediger, “Apparatus and method for preparative gel electrophoresis,” Anal. Biochem., vol. 142, no. 2, pp. 445–454, Nov. 1984, doi: 10.1016/0003-2697(84)90488-3.

[15] G. Carvalho et al., “Bacterial Transformation Buffers Environmental Fluctuations through the Reversible Integration of Mobile Genetic Elements,” mBio, Apr. 2020, doi: 10.1128/mBio.02443-19.

[16] E. R. Sanders, “Aseptic laboratory techniques: plating methods,” J. Vis. Exp. JoVE, no. 63, p. e3064, May 2012, doi: 10.3791/3064.

[17] R. E. Huber, J. Lytton, and E. B. Fung, “Efflux of beta-galactosidase products from Escherichia coli,” J. Bacteriol., vol. 141, no. 2, pp. 528–533, Feb. 1980, doi: 10.1128/jb.141.2.528-533.1980.

[18] A. Bock and G. Sawers, “Escherichia coli and Salmonella: Cellular and molecular biology, 2nd edition,” Fermentation, pp. 262–282, Jan. 1996.

[19] S. Oehler, E. R. Eismann, H. Krämer, and B. Müller-Hill, “The three operators of the lac operon cooperate in repression.,” EMBO J., vol. 9, no. 4, pp. 973–979, Apr. 1990.

[20] “MacConkey-Agar-Plates-Protocols.pdf.” Accessed: Feb. 27, 2022. [Online]. Available: https://asm.org/ASM/media/Protocol-Images/MacConkey-Agar-Plates-Protocols.pdf?ext=.pdf

[21] B. Jung and G. J. Hoilat, “MacConkey Medium,” in StatPearls, Treasure Island (FL): StatPearls Publishing, 2022. Accessed: Feb. 27, 2022. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK557394/

[22] “IB Chemistry higher level notes: Indicators,” Sep. 22, 2009. https://web.archive.org/web/20090922042431/https://ibchem.com/IB/ibnotes/full/aab_htm/18.6.htm (accessed Feb. 27, 2022).

[23] “Lactic Acid - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/lactic-acid (accessed Feb. 27, 2022).

[24] J. Pearson, M. S. Ganio, T. Seifert, M. Overgaard, N. H. Secher, and C. G. Crandall, “Pulmonary Artery and Intestinal Temperatures during Heat Stress and Cooling,” Med. Sci. Sports Exerc., vol. 44, no. 5, pp. 857–862, May 2012, doi: 10.1249/MSS.0b013e31823d7a2b.

[25] M. Cheng et al., “An engineered genetic circuit for lactose intolerance alleviation,” BMC Biol., vol. 19, no. 1, p. 137, Jul. 2021, doi: 10.1186/s12915-021-01070-9.

[26] “Life Technologies - CA.” //www.thermofisher.com/ca/en/home.html (accessed Feb. 28, 2022).

[27] “BioBrick® Assembly | NEB.” https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/biobrick-assembly (accessed Feb. 28, 2022).

[28] A. Szilagyi, “Chapter 2 - Digestion, absorption, metabolism, and physiological effects of lactose,” in Lactose, M. Paques and C. Lind

[29] “parts.igem.org.” http://parts.igem.org/Main_Page (accessed Feb. 28, 2022).

[30] E. M. Ozbudak, M. Thattai, H. N. Lim, B. I. Shraiman, and A. Van Oudenaarden, “Multistability in the lactose utilization network of Escherichia coli,” Nature, vol. 427, no. 6976, pp. 737–740, Feb. 2004, doi: 10.1038/nature02298.

[31] “Reference Links for Key Numbers in Biology.” https://bionumbers.hms.harvard.edu/keynumbers.aspx (accessed Mar. 24, 2022).

[32] C. Harrison *et al.*, “Comparison of Kinetic Models for Analysis of Pyruvate-to-Lactate Exchange by Hyperpolarized 13C NMR,” *NMR Biomed.*, vol. 25, no. 11, pp. 1286–1294, Nov. 2012, doi: 10.1002/nbm.2801.

[33] S. Semsey et al., “The effect of LacI autoregulation on the performance of the lactose utilization system in Escherichia coli,” Nucleic Acids Res., vol. 41, no. 13, pp. 6381–6390, Jul. 2013, doi: 10.1093/nar/gkt351.

[34] N. Yildirim and M. C. Mackey, “Feedback Regulation in the Lactose Operon: A Mathematical Modeling Study and Comparison with Experimental Data,” Biophys. J., vol. 84, no. 5, pp. 2841–2851, May 2003.

[35] M. Du, S. Kodner, and L. Bai, “Enhancement of LacI binding in vivo,” Nucleic Acids Res., vol. 47, no. 18, pp. 9609–9618, Oct. 2019, doi: 10.1093/nar/gkz698.

[36] J. A. Bernstein, A. B. Khodursky, P.-H. Lin, S. Lin-Chao, and S. N. Cohen, “Global analysis of mRNA decay and abundance in Escherichia coli at single-gene resolution using two-color fluorescent DNA microarrays,” Proc. Natl. Acad. Sci., vol. 99, no. 15, pp. 9697–9702, Jul. 2002, doi: 10.1073/pnas.112318199.

[37] A. Kamionka, J. Bogdanska-Urbaniak, O. Scholz, and W. Hillen, “Two mutations in the tetracycline repressor change the inducer anhydrotetracycline to a corepressor,” Nucleic Acids Res., vol. 32, no. 2, pp. 842–847, 2004, doi: 10.1093/nar/gkh200.

[38] “Team:HSiTAIWAN/Model - 2016.igem.org.” http://2016.igem.org/Team:HSiTAIWAN/Model (accessed Mar. 24, 2022).

[39] P. García, V. Ladero, J. Alonso, and J. Suárez, “Cooperative Interaction of CI Protein Regulates Lysogeny of Lactobacillus casei by Bacteriophage A2,” J. Virol., vol. 73, pp. 3920–9, Jun. 1999, doi: 10.1128/JVI.73.5.3920-3929.1999.

[40] M. Martinez-Bilbao, R. E. Holdsworth, L. A. Edwards, and R. E. Huber, “A highly reactive beta-galactosidase (Escherichia coli) resulting from a substitution of an aspartic acid for Gly-794.,” J. Biol. Chem., vol. 266, no. 8, pp. 4979–4986, Mar. 1991, doi: 10.1016/S0021-9258(19)67745-8.

[41] L. Li, Y. Liu, W. Wang, J. Cheng, W. Zhao, and P. Wang, “A highly efficient galactokinase from Bifidobacterium infantis with broad substrate specificity,” Carbohydr. Res., vol. 355, pp. 35–39, Jul. 2012, doi: 10.1016/j.carres.2012.04.022.

[42] J. E. Tuininga, C. H. Verhees, J. van der Oost, S. W. M. Kengen, A. J. M. Stams, and W. M. de Vos, “Molecular and Biochemical Characterization of the ADP-dependent Phosphofructokinase from the Hyperthermophilic Archaeon Pyrococcus furiosus,” J. Biol. Chem., vol. 274, no. 30, pp. 21023–21028, Jul. 1999, doi: 10.1074/jbc.274.30.21023.

[43] J. L. Powers, N. E. Kiesman, C. M. Tran, J. H. Brown, and V. L. H. Bevilacqua, “Lactate dehydrogenase kinetics and inhibition using a microplate reader,” Biochem. Mol. Biol. Educ., vol. 35, no. 4, pp. 287–292, 2007, doi: 10.1002/bmb.74.

[44] M. J. Reddish, H.-L. Peng, H. Deng, K. S. Panwar, R. Callender, and R. B. Dyer, “Direct Evidence of Catalytic Heterogeneity in Lactate Dehydrogenase by Temperature Jump Infrared Spectroscopy,” J. Phys. Chem. B, vol. 118, no. 37, pp. 10854–10862, Sep. 2014, doi: 10.1021/jp5050546.

[45] C. Harrison et al., “Comparison of Kinetic Models for Analysis of Pyruvate-to-Lactate Exchange by Hyperpolarized 13C NMR,” NMR Biomed., vol. 25, no. 11, pp. 1286–1294, Nov. 2012, doi: 10.1002/nbm.2801.