Modern Biology Cat # IND 28

STUDENT MANUAL

"Synthetic Biology: Using Bacterial Computers to Solve the Pancake Problem"

INTRODUCTION

Defining Synthetic Biology

Synthetic biology is an exciting new field that uses engineering principles and mathematical modeling to design and construct biological devices with applications in energy, medicine, environmental studies, and technology (Endy, 2005). The blurring of traditional disciplines that occurs in synthetic biology – between biology, computer science, mathematics, and engineering; and between education and research – promises to catalyze widespread reform of scientific research.

Recombinant DNA technology is the technical foundation of synthetic biology and has matured over the past thirty-five years. Advances in our ability to synthesize DNA, cut and paste it with enzymes, amplify it by PCR, sequence it, and introduce it into cells have made molecular cloning not only more versatile, but extremely accessible and increasingly affordable. However, synthetic biology is more than a collection of experimental tools; synthetic biology is a way of thinking that uses fundamental principles of engineering to promote collaboration and creativity (Chopra and Kamma, 2006; Campbell, 2005). One fundamental engineering principle is standardization. Those of us who are not engineers take for granted that there are collections of construction materials, such as nuts and bolts, which are standardized, catalogued, quality-controlled, and have well-defined properties agreed upon by a community (Knight, 2003). Standardization of parts is accompanied by the standardization of the assembly of those parts; nuts and bolts go together in ways that are agreed upon by the entire world. Standardization creates efficiencies and encourages collaboration. Another important engineering principle is abstraction. Abstraction allows engineers to manage complexity, to specialize, and to engage in creative and higher order thinking. Consequently, parts made from basic materials are assembled into devices, which in turn are combined to produce systems. Raw materials of metal, plastic and silicon are used to make microprocessor parts that are assembled into a hard drive device, which is then integrated into the system of a computer.

In synthetic biology, standardization of parts is occurring in much the same way it did in mechanical engineering. The community of synthetic biologists is coming to agreement on what properties biological parts should have and how their functions should be measured. The most extensive collection of DNA parts is the Registry of Standard Biological Parts, a catalog of over 5,000 protein-coding sequences, promoters, ribosome binding sites, transcriptional terminators, and many other useful components of genetic circuits (http://partsregistry.org/Main_Page). The Registry parts are designed as "BioBricks," enabling their assembly in a standardized fashion using a clever scheme

(Knight, 2003). The result of putting two BioBrick parts together produces a new BioBrick composite part, analogous to the assembly of legos. DNA is the raw material used to construct biological parts, designing them from scratch or borrowing them from genomes that occur in nature. The DNA parts are assembled into devices that are in turn used to construct systems. Synthetic biologists also use the engineering principle of abstraction. Abstraction simplifies thinking about complex systems and encourages creativity in the process of engineering living cells. A synthetic biologist does not have to know the DNA sequence of parts to use them. Similarly, a user does not have to understand how a part works as long as the behavior of the part is characterized. Because systems function in ways that cannot always be predicted by the functions of their constituent parts and devices, abstraction is reminiscent of emergent properties in biological systems.

Research in synthetic biology most often follows one of two paths. One path is to use the tools and ways of thinking of synthetic biology in a reductionist approach to understanding natural biological systems. By deconstructing complex systems into devices and their component parts, and reassembling them, synthetic biologists can develop and test hypotheses about how natural systems function. They can compare the behavior of their synthetic models to natural systems and devise experiments to learn why differences occur. Another path is to borrow parts and devices from nature, or design and construct synthetic ones, and assemble systems that enable living cells to carry out new functions not found in nature. In this way, synthetic biologists seek to compile a collection of components and develop the tools needed to engineer biology. However, these two paths are not mutually exclusive because by building a synthetic device, scientists can learn how natural systems function.

Early successes in synthetic biology have captured worldwide attention. For example, Chris Voigt and his group engineered bacteria to respond to the anaerobic environment produced by cancerous cells by secreting a cytotoxin that can kill cancer cells (Anderson, *et al,* 2006). The study was a first step toward arming bacteria that can sense the microenvironment of a tumor and selectively kill it. Michael Elowitz at Caltech constructed the “represillator,” a synthetic oscillatory network consisting of three interacting repressors in a negative feedback loop (Elowitz and Leibler, 2000). At the University of California, Berkeley, Jay Keasling and his group used synthetic biology to re-engineer the metabolism of bacteria and yeast to produce artemisinin, an important antimalarial drug (Dae-Kyun *et al.*, 2006). Keasling was named the 2006 *Discover Magazine* scientist of the year in part because he reduced the cost of malaria medication by a factor of ten. These and other examples of successful synthetic biology applications serve to invigorate the existing synthetic biology community and encourage others to join it (GCAT, 2010). Early successes validate the synthetic biology approach as a means to understand complex natural biological systems and to design artificial ones with important applications.

Interaction of Mathematics and Synthetic Biology

The field of synthetic biology is providing great opportunities for students who enjoy several subjects, especially mathematics and biology, to work as part of interdisciplinary teams. In addition to standardization and abstraction, another fundamental principle of synthetic biology is mathematical modeling. Traditional molecular biology rarely utilized modeling when designing DNA constructs, but relied instead on selection of DNA constructs exhibiting phenotypes that approximate the

desired outcome. However, this selection-based approach does not allow the biologist to predict outcomes or determine if component parts have failed to perform as expected. Furthermore, modeling allows the biologists to use their time more wisely by building only DNA devices that have a reasonable chance of working rather than building hundreds of versions and selecting the few that work. Consistent with the more efficient use of lab time is an expression that says, “Six months in the lab can save you an afternoon of modeling on the computer.”

One example of a synthetic biology project that has blended mathematics, computer science and biology was the construction of bacterial computers that can solve mathematical problems (Haynes, *et al.*, 2008; Baumgardner, *et al.* 2009). Imagine a computer so small that a billion of them fit in 1 mL of liquid. Now imagine that same computer replicating itself every 20 minutes. It sounds like science fiction, but microbial machines, in the form of genetically engineered *E. coli* cells, have solved interesting mathematical problems. The exponential growth of bacterial cells and the ability to replicate genetic computer programs within those cells, gives hope that a biological computer may someday be able to address intractable real-world problems. Traditional computers work serially, completing one step before moving to the next, but a biological approach to a mathematical problem could provide parallel processing on a scale unimaginable with silicon computers.

The Burnt Pancake Problem (BPP)

The experiments and exercises you are about to perform use the principles of standardization, abstraction and mathematical modeling. You are going to explore and learn about the use of bacterial computers to solve a simple version of the burnt pancake problem (BPP). The BPP can be visualized as a stack of delicious pancakes, each of a different size and having one burnt side and one golden side, arranged in an arbitrary order (Figure 1A). The stack can be rearranged by flipping individual pancakes or several adjacent pancakes. The goal is to sort the pancakes from smallest to largest with each pancake oriented golden side up. The challenge of the BPP is to sort the stack in the fewest possible flips. Mathematically, the BPP is also known as “sorting by reversals” since both the order and orientation of the pancakes are reversed when they are flipped. However, the BPP is a more than a puzzle. Mathematicians and computer scientists are interested in studying how many flips it takes to solve the BPP, and in developing algorithms (step by step procedures that can be programmed in a computer) to solve it. The only academic publication by Bill Gates, of Microsoft fame, described the version of the pancake problem that you will study. Biologists are interested in the application of the BPP to genomics, too. For example, mice and humans have very similar genes on their chromosomes, but the order and orientation of many genes have been reversed, or flipped (Figure 1B). One way to measure the evolutionary distance between two organisms is to find the minimum number of reversals required to sort regions of genes in one organism to match the order and orientation of equivalent genes in the other organism. Therefore, the computer science challenge and the biological question converge in the BPP.

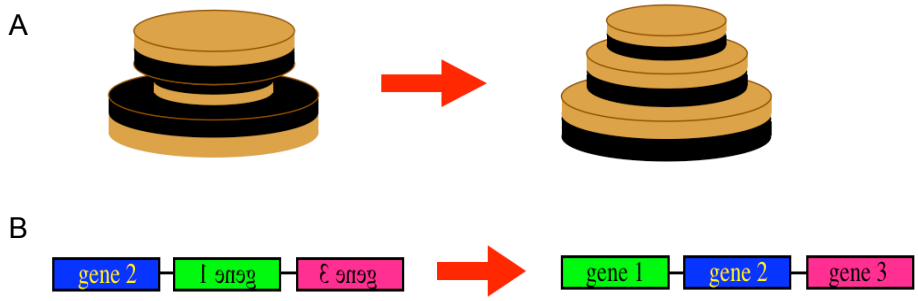


Figure 1. The Burnt Pancake Problem. A. A starting stack of pancakes on the left and the solution on the right. B. Using genes to represent the pancakes. The order of genes on the left is the same as the order of pancakes on the left of panel (A). The order of genes on the right is the same as the order of pancakes on the right of panel (A).

In the experiment you are about to perform, the biological equivalent of a burnt pancake is a functional module of DNA such as a promoter or coding region (Figure 2). Similar to burnt pancakes in the BPP, DNA modules have directionality (5' to 3'), require a specific order of the units (*e.g.*, promoter followed by coding region) and can be flipped (genetic recombination of cut, invert, and splice by cellular machinery). The bacterial computer you will be investigating uses a modular system in which pancake stacks are assembled from flippable DNA segments. Flipping of the DNA segment "pancakes" is performed by a naturally occurring recombination system called Hin/hix (described below), which has been reengineered by synthetic biologists and provided to you in a plasmid. The Hin/hix system is an example of abstraction because you don't need to know how it works to understand how it is used to solve the BPP in living cells that function as biological computers.

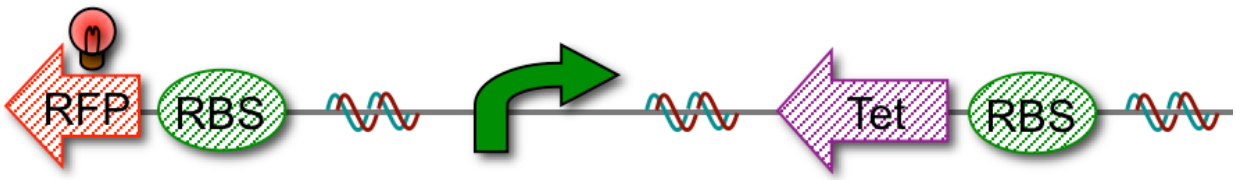


Figure 2. Starting configuration of the burnt pancake bacterial computer. The double helix figures indicate three hix recombination sites. DNA between two hix sites define a pancake containing a transcriptional promoter (arrow) and a second pancake with a ribosome binding site (RBS) and a tetracycline resistance gene (Tet). Since the first pancake faces forward and the second backwards, the configuration can be described as (1,-2).

Genetic Recombination and the Hin/hix System

The Hin/hix system that you will be using to solve the BPP is an example of genetic recombination, the important process by which cells reshuffle genetic material. Genetic recombination is fundamental to evolution, since it produces genetic variation that can be acted upon by natural selection. Recombinases are enzymes found in all living cells that bind to DNA, cleave the phosphate-sugar backbone, and reassemble it in a new configuration. The Hin recombinase serves the purpose of providing variation of flagellar proteins for *Salmonella* bacteria, allowing them to evade an immune response. The recombination reaction is initiated when Hin binds to a pair of 26 bp *hix* sites in the substrate DNA. In an energy-dependent reaction, Hin catalyzed the cleavage and rejoining of both strands of the DNA, resulting in flipping of the DNA sequence between the *hix* sites. The Hin/hix system was adapted for use in the biological computer that you will be using to study the Burnt Pancake Problem.

REFERENCES

Anderson JC, et al. 2006. Environmentally controlled invasion of cancer cells by engineered bacteria. *J Mol Biol.* Vol. 355(4): 619-27.

Baumgardner, Jordan, *et al.* 2009. Solving a Hamiltonian Path Problem with a Bacterial Computer. *Journal of Biological Engineering*. Vol. 3:11.

Campbell AM. 2005. Meeting Report: Synthetic Biology Jamboree for Undergraduates. *Cell Biology Education*. Vol. 4(1): 19-23.

Chopra P and Kamma A. 2006. Engineering Life through Synthetic Biology. *In Silico Biology* 6, 401.

Dae-Kyun Ro, Eric M Paradise, *et al*. 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature. Vol. 440: 940-943.

Elowitz M and Leibler S. 2000. A Synthetic Oscillatory Network of Transcriptional Regulators. *Nature*. Vol. 403: 335-338.

Endy D. 2005. Foundations for Engineering Biology. *Nature.* Vol. 438: 449-453.

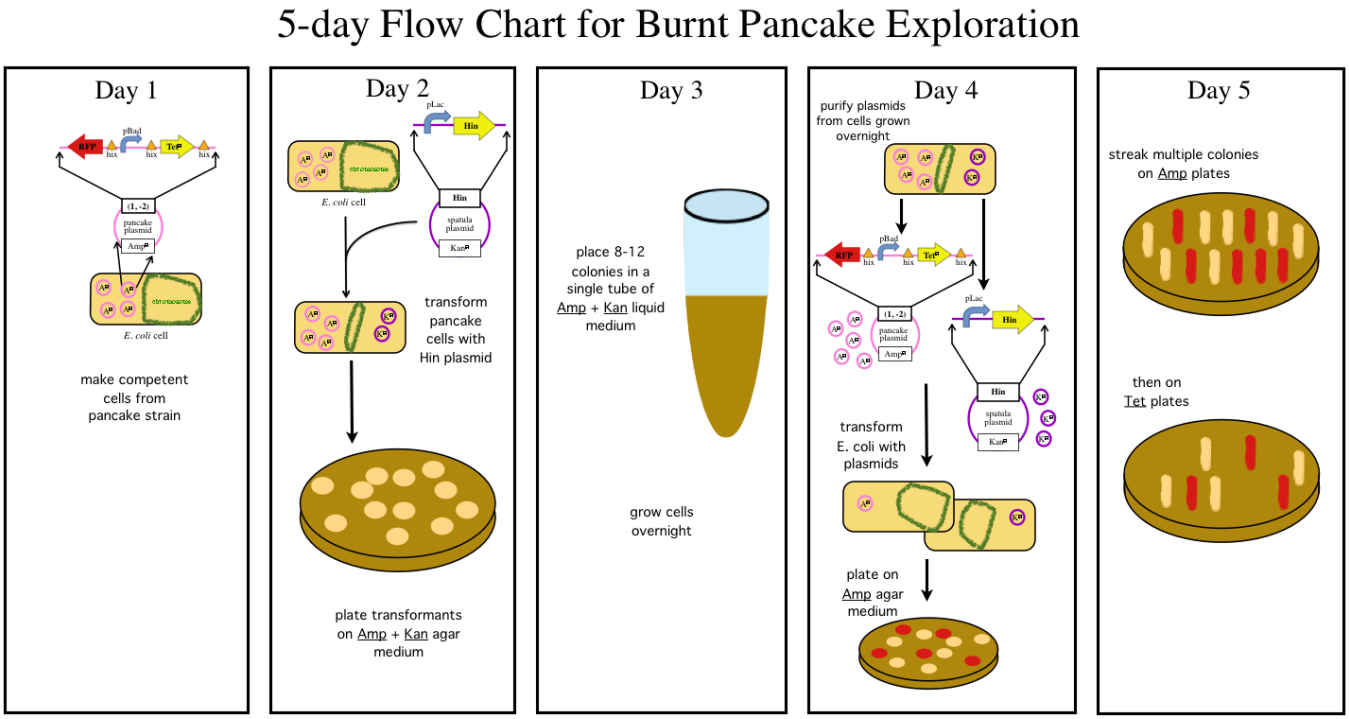
GCAT. 2010. What is Synthetic Biology? (http://www.bio.davidson.edu/projects/GCAT/Synthetic/What_Is_SynBio.html). Accessed 27 July, 2010.

Haynes, Karmella A., *et al.* 2008. Engineering bacteria to solve the Burnt Pancake Problem. *Journal of Biological Engineering*. Vol. 2(8): 1 – 12.

Knight TF. 2003. Idempotent Vector Design for Standard Assembly of BioBricks. *Tech. rep., MIT Synthetic Biology Working Group Technical Reports.*

EXPERIMENTAL PROCEDURE

The first goal in the investigation is to establish a population of bacterial computers that can begin working on the BPP. The procedure begins with a bacterial clone (called "Pancake") that contains a single configuration (see Figure 2) of the two pancakes (1, -2) on a plasmid that carries ampicillin resistance. The starting configuration will be recombined by exposure to the Hin recombinase, and will produce a distribution of pancake configurations. Below is a flowchart of the experiments you will conduct.



Day 1 – **Preparation of competent cells from Pancake strain**-These steps should be carried out by the instructor before the laboratory period.

1. Place the vial of $CaCl_2$ and the tube of *E. coli* "Pancake" cells in the ice bath.

2. Using a sterile pipet, transfer about 1/2 ml of the $CaCl_2$ solution to the tube containing the bacteria.

3. Using the same pipet, transfer the contents of this tube back into the larger vial that contains most of the $CaCl_2$ solution.

4. Tap the vial with the tip of your index finger to mix the solution.

5. Incubate the cells for about 3-18 hours on ice. The cells are then called competent because they can take up DNA from the medium. The cells will settle to the bottom of the vial. In order to concentrate the cells, remove and discard about half of the $CaCl_2$ solution from the top portion of the vial. The cells are now ready for transformation.

Question: What is the expected combination of phenotypes produced by the (1, -2) Pancake clone? Are cells with this plasmid expected to glow red? Are they expected to be resistant to tetracycline?

Day 2 – **Transform Pancake cells with Hin plasmid and plate transformants on Amp + Kan agar medium**

1. Label a 1.5 ml sterile tube "Hin" and use a sterile micropipette to transfer 10 μL of the Hin plasmid into it. Place the tube in an ice bath.

2. Gently tap the vial of "Pancake" competent cells with the tip of your index finger to ensure that the cells are in suspension. Then, using a sterile pipet, add 100 μL of the competent cells to the plasmid tube from step 1 above. Gently tap the tube with the tip of your index finger to mix these solutions and store the tube on ice for 30 - 60 minutes.

3. Transfer the tube to a water bath, preheated to 37°C, for 5 minutes. This heat shock facilitates the uptake of plasmid DNA.

4. Add 0.4 mL of nutrient broth to the tube and incubate at 37°C for 30 minutes. The nutrient broth should be dispensed with a sterile transfer pipet. This incubation period allows the bacteria time to recover from the $CaCl_2$ treatment and to begin to express the kanamycin-resistance gene on the Hin plasmid.

5. Using a sterile pipet, remove 0.25 mL of the mixed bacterial suspension from the tube, remove the lid from a nutrient agar plate containing amplicillin + kanamycin and dispense the bacteria onto the agar. Spread the bacteria evenly onto the agar surface using a sterile bent glass rod. Your instructor will demonstrate this procedure.

6. Replace the lid on the plate and leave it at room temperature until the liquid has been absorbed (about 10-15 minutes).

7. Invert the plate and incubate at 37°C for 16-18 hours.

Question: What is the purpose of plating the Pancake cells on media with two different antibiotics?

Day 3 – Place 8-12 colonies in a single tube of Amp + Kan liquid medium and grow cells overnight

Observation: What color are the colonies? Observe them on a light box or overhead projector. Put on a pair of safety glasses and shine a handheld UV light on the colonies in a darkened room (CAUTION: UV light is harmful to eyes, so be sure to use safety glasses). Do any of the colonies fluoresce under UV light?

1. Transfer 8-12 colonies from the plate to a single tube that contains 5 mL of liquid media with ampicillin and kanamycin and incubate at 37°C with shaking overnight.

Question: Based on what you have learned about the Hin/hix recombination system, what do you think will happen to the original (1,-2) pancake configuration during growth in media with ampicillin and kanamycin?

Day 4 - Purify plasmids from cells grown overnight, transform E. coli with plasmids, and plate on Amp agar medium

The next step is to purify the population of "Pancake" plasmids from the overnight bacterial culture. This plasmid preparation will actually yield both the "Pancake" and "Hin" plasmids. However, you will introduce the plasmids into competent bacteria while selecting for only the Ampicillin resistant "Pancake" plasmids. During this process, each resulting bacterial transformant will initially receive one plasmid, which will be replicated into several hundred plasmids per cell in a colony of cloned bacteria. Any given cell only takes up one copy of a single plasmid.

Plasmid Isolation

1. Transfer about 1.5 ml of the culture to a labeled 1.5 ml microcentrifuge tubes and centrifuge at maximal speed for 1-2 minutes to pellet the bacteria.

2. Discard the supernatant, fill the tube again with the culture and centrifuge for 1-2 minutes. Repeat this step one time.

3. Carefully remove and discard the supernatant fluid. Invert the tube and allow the cell pellet to dry for a minute.

4. Add 250 ul of Solution I to the tube and suspend the pellet in the solution by vigorous shaking or by using a vortex mixer. This solution contains RNAse, which destroys RNA. Complete re-suspension (no visible clumps) is important in order to have high plasmid yield.

5. Incubate the tube at room temperature for 2 minutes.

6. Add 250ul of Solution II, cap the tube and mix by inversion several times until the contents are mixed thoroughly. The solution should become clear and viscous within 1-2 minutes. Solution II contains the detergent sodium dodecyl sulfate (SDS) and sodium hydroxide, which dissolve the bacterial membranes and causes cell lysis. The sodium hydroxide also denatures the DNA. Upon neutralization in the step below, the plasmid DNA strands renature because of their proximity while the bacterial DNA does not.

7. Add 350 ul of Solution III to the tube, cap the tube and shake until the contents are mixed thoroughly. A white flocculent precipitate should form. The acetic acid in Solution III will neutralize the solution. The Guanidium Chloride in Solution III and the SDS from the previous step produce a tangled network of long strands of

bacterial chromosomal DNA and cell debris, and this matrix can be separated from the smaller plasmid DNA by centrifugation.

8. Centrifuge the tube for 10 minutes at maximal speed. After centrifugation, plasmid DNA and small amounts of cellular proteins are in the supernatant, and bacterial chromosomal DNA and most of the proteins are in the pellet. This supernatant is known as a "cleared lysate". A common procedure that is used to remove the proteins, SDS and salts that are present in the cleared lysate involves the use silica, which binds to the DNA but not to the contaminants. The silica is packed into the bottom of a column that you will use, and the lysate is added to the column over the silica. The column is then washed by centrifugation to remove the contaminants and the plasmid DNA is eluted by using a low salt buffer.

9. Obtain a blue column, and a collection tube, label each, and insert the column into the collection tube. Add 100 ul of Equilibration Buffer to the column and centrifuge for 1 minute at maximal speed.

10. Carefully pour the cleared lysate from step 5 into the column making sure that the pellet is not dislodged. Centrifuge the column with collecting tube for 1 minute at maximal speed.

11. Discard the liquid in the collection tube, place the column back into the collection tube and add 500 ul of HB Buffer to the columns. Centrifuge the column/collecting tube for 1 minute.

12. Discard the liquid in the collection tube, place the column back into the collection tube and add 700 ul of Wash Buffer to the column. Centrifuge the column/collecting tube for 1 minute.

13. Discard the liquid in the collection tube, place the column back into the collection tube and centrifuge for 2 minutes to dry the column.

14. Discard the collection tube and insert your column into a clean-labeled 1.5-2 ml centrifuge tube. Place 30 ul of Elution Buffer onto the center of the silica in the column and incubate for 2 minutes. Centrifuge for 2 minutes, discard the column and cap the tube that contains the eluted purified plasmid DNA. The plasmid DNA can be stored in the freezer or processed as described below.

Preparation of Competent Cells-These steps should be carried out by the instructor before the laboratory period.

1. Place the vial of $CaCl_2$ and the tube of *E. coli* "Pancake" cells in the ice bath.

2. Using a sterile pipet, transfer about 1/2 ml of the $CaCl_2$ solution to the tube containing the bacteria.

3. Using the same pipet, transfer the contents of this tube back into the larger vial that contains most of the $CaCl_2$ solution.

4. Tap the vial with the tip of your index finger to mix the solution.

5. Incubate the cells for about 1-18 hours on ice. The cells are then called competent because they can take up DNA from the medium. The cells will settle to the bottom of the vial. In order to concentrate the cells, remove and discard about half of the $CaCl_2$ solution from the top portion of the vial.

Transformation

1. Label a 1.5 mL sterile tube for each plasmid preparation and transfer 5 μL of the plasmid DNA into the tube.

2. Gently tap the vial of "Pancake" competent cells with the tip of your index finger to ensure that the cells are in suspension. Then, using a sterile pipet, add 100 μL of the competent cells to each plasmid tube. Tap each of these tubes with the tip of your index finger to mix these solutions and store the tubes on ice for 30 - 90 minutes.

3. Transfer the tubes to a water bath, preheated to 37°C, for 5 minutes. This heat shock facilitates the uptake of plasmid DNA.

4. Add 0.4mL of nutrient broth to each tube and incubate at 37°C for 30 minutes. The nutrient broth should be dispensed with a sterile transfer pipet. This incubation period allows the bacteria time to recover from the $CaCl_2$ treatment and to begin to express the ampicillin-resistance gene on the Hin plasmid.

5. Using a sterile pipet, remove 0.2mlL of the mixed bacterial suspension from the tube, remove the lid from a nutrient agar plate containing amplicillin and dispense the bacteria onto the agar. Spread the bacteria evenly onto the agar surface. Using a sterile bent glass rod.

6. Replace the lids on the plates and leave the plates at room temperature until the liquid has been absorbed (about 10-15 minutes).

7. Invert the plates and incubate at 37° C for 16-18 hours.

Question: What will happen to the kanamycin resistance plasmids during the transformation and selection? What will be the effect of the introduction of only one pancake plasmid to each competent bacterial cell?

Day 5 and 6 – Streak multiple colonies on Amp plates, then on Tet plates

1. Remove the plate from the incubator.

   Observation: What color are the colonies? Observe them on a light box or overhead projector. Put on a pair of safety glasses and shine a handheld UV

light on the colonies in a darkened room (CAUTION: UV light is harmful to eyes, so be sure to use safety glasses). You may or may not see red colonies at this time. The step below will help you to identify the bacteria expressing RFP.

2. In order to more clearly identify RFP producing cells, obtain an agar plate containing amplicillin and mark #1-20 on the bottom of the plate with a marker pen.

2. Transfer 20 randomly chosen well isolated colonies from your plates as ~1 cm streaks onto the plate. Use toothpicks fresh from a box for the transfers. Incubate the plate overnight at 37° C.

Question: Is it possible for a given cell to express both the red fluorescence and tetracycline resistance phenotypes?

Observation:

The red color should now be easily seen without a UV lamp. Record in the Table below the color of each streak.

Day 6

Obtain an agar plate containing tetracycline and mark #1-#20 on the bottom of the plate with a marker pen. Transfer bacteria from each of the 20 streaks as ~1 cm streaks onto this agar plate. Use toothpicks fresh from a box for the transfers. Incubate the plate overnight at 37° C.

Observation: Did all the streaks grow on the tetracycline plate? Add your observation of resistance or sensitivity to tetracycline to the color/fluorescence phenotype for each clone in the Table below.

Question: Results from living cells do not always match our theoretical expectations, causing us to reconsider. How would our phenotypic predictions change if the promoter has backwards activity?

Table 1. Summary of the Analysis

Streak Number	+/- Red Fluorescence	+/- Tetracycline Resistance	Streak Number	+/- Red Fluorescence	+/- Tetracycline Resistance
1.			11.		
2.			12.		
3.			13.		
4.			14.		
5.			15.		
6.			16.		
7.			17.		
8.			18.		
9.			19.		
10.			20.		

MATHEMATICAL INVESTIGATIONS

Day One

PLAYING WITH PARTS

1. To become more familiar with the BPP, experiment with cutout icons for the promoter and the protein coding region. Find and sketch all the possible arrangements of the two biological burnt pancakes.

2. To make a functional gene, the promoter must precede the protein coding portion (RBS + Reporter), and the promoter and the protein coding portion must face the same direction. How many of the arrangements you found in #1 will make a functional reporter gene?

Day Two

INTRODUCTION TO PERMUTATIONS

To mathematically represent the BPP and our DNA constructs, we need a notation that captures the information of both order and orientation. We begin with the mathematical notion of permutations which address order only. Formally, a permutation is a one-to-one function from a set {1,2,…,*n*} onto itself. Less formally, a permutation is an ordering of the number 1,2,…,*n.* One convenient notation for permutations is to present the information as an ordered *n*-tuple. For example, (3,1,2) denotes the arrangement of the three objects 1, 2, and 3 so that 3 is first, followed by 1, followed by 2. The questions below will help you to think about permutations and their relation to a variation of the BPP which can be thought of as the stacking of unburned pancakes with no distinguishable top or bottom. You can think of the numbers as representing pancakes of distinct sizes numbered 1,2,…,*n* by increasing size and the ordered *n*-tuple as listing the pancakes in order from top to bottom of the stack.

Investigation Questions:

1. In how many ways can 2 unburned pancakes be stacked? List them using permutation notation.

2. In how many ways can 3 unburned pancakes be stacked? List them.

3. In how many ways can 4 unburned pancakes be stacked? (List them only if necessary for you to answer the counting question.)

4. Find a pattern and give a formula for the number of ways that any number *n* of unburned pancakes can be stacked.

5. Suppose the initial starting positions of three objects is (1,2,3). How many of the permutations yield the result in which none of the three objects ends up in the same position that it started? List the permutations that leave no object in its original position. Such permutations are called *derangements*.

6. For four objects and starting position (1,2,3,4), list all of the derangements.

7. Estimate the number of derangements of five objects.

Day Three

INTRODUCTION TO SIGNED PERMUTATIONS

For the BPP, we need a notation that captures the information of both order and orientation. To add the information about orientation, we can use a negative sign to indicate that a pancake is burnt side up. For example, (3,-1,-2) denotes that the 3 is first and in forward orientation (the largest pancake is on top with golden side up), followed by the 1 in backward orientation (the smallest pancake with burnt side up), followed by 2 in backward orientation (the medium pancake with burnt side up). This notation is called a *signed permutation*, and is useful to think about both the pancake stacks and the genetic circuit diagrams. In what follows, we will consider the pancakes distinct sizes numbered from 1 to *n* in increasing size. These questions differ from those from Day 2 in that these involve burnt pancakes which have orientation in addition to order.

Investigation Questions:

1. In how many ways can 2 burnt pancakes be stacked? List them using signed permutation notation.

2. In how many ways can 3 burnt pancakes be stacked?

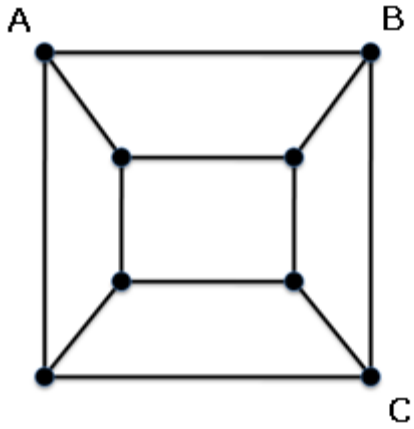
3. In how many ways can 4 burnt pancakes be stacked?

4. Find a pattern and give a formula for the number of ways that any number *n* of burnt pancakes can be stacked.

Day Four

INTRODUCTION TO PANCAKE GRAPHS

The word *graph* is used in many ways in mathematics. Certainly you are familiar with the graph of a function on an *xy*-coordinate system. Another use of the word graph in mathematics is a diagram that conveys the relationships between a set of objects. Formally, a graph (for our purposes here) is a non-empty collection of points (called *vertices*) together with edges that connect some (or all or none) pairs of points. Two vertices with an edge between are called *adjacent*. For the graph below, vertex A is adjacent to vertex B, and vertex B is adjacent to vertex C, but vertex A is not adjacent to vertex C because there is no single edge that connects them.

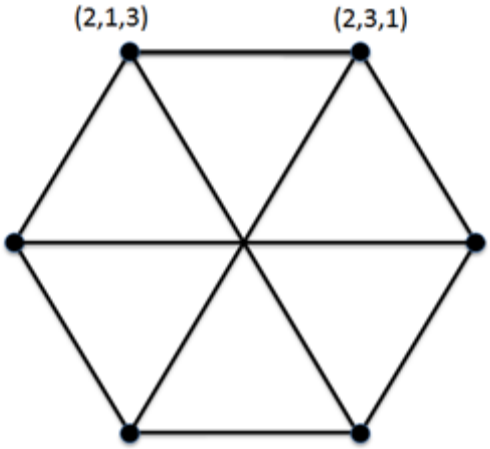


For our first exercises, we look what are called permutations and permutation graphs. Specific to our investigation here, we represent each of the signed permutations corresponding to a stack of unburned or burnt pancakes and we connect two of these signed permutations with an edge if it is possible to get from one permutation to the other by changing the order of one or more adjacent pancakes in the stack. We will call such a graph a *pancake graph*. For example, for three unburned pancakes, there would be an edge between (2,1,3) and (2,3,1) because inverting the second and third pancakes takes us from one of these permutations to the other.

For three burnt pancakes, beginning with (2,1,3) and inverting the second and third pancakes results in (2,-3,-1).

Investigation Questions:

1. In the unburned pancake graph below with three pancakes, list all permutations that are adjacent to (2,3,1).



2. Above is the pancake graph for three pancakes with the vertices (2,1,3) and (2,3,1) labeled. Label the other vertices with so that the edges correspond to potential flips.

3. How many permutations are adjacent to both (2,1,3) and (1,2,3)?

4. If we define the *distance* between two permutations as the number of edges in the shortest path that connects one to the other, how many vertices are of distance 2 from (2,1,3) in your unburned pancake graph with three pancakes? List them.

5. List all vertices in the graph that are of distance 3 from (2,1,3).

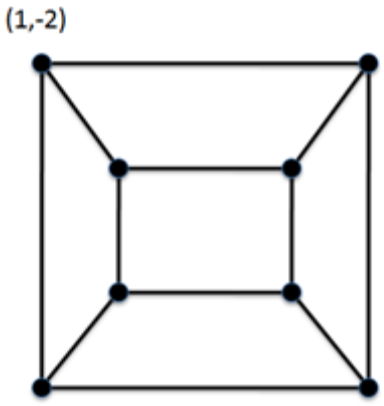
Day Five

INTRODUCTION TO BURNT PANCAKE GRAPH

Specific to our investigation here, we represent each of the signed permutations corresponding to a stack of unburned or burnt pancakes and we connect two of these signed permutations with an edge if it is possible to get from one permutation to the other by changing the order of one or more adjacent pancakes in the stack. We will call such a graph a *pancake graph*. For example, for three unburned pancakes, there would be an edge between (2,1,3) and (2,3,1) because inverting the second and third pancakes takes us from one of these permutations to the other. For three burnt pancakes, beginning with (2,1,3) and inverting the second and third pancakes results in (2,-3,-1).

Investigation Questions:

1. In the burnt pancake graph for two pancakes, list all permutations that are adjacent to (1,-2).



2. Above is the burnt pancake graph for two pancakes with the vertex (1,-2) labeled. Label the other vertices with so that the edges correspond to potential flips. (There is more than one possible answer.)

3. In the burnt pancake graph with two pancakes, how many permutations are adjacent to both (1,-2) and (-1,2)? adjacent to both (1,-2) and (1,2)?

4. How many vertices are of distance 2 from (1,-2) in your burnt pancake graph with two pancakes? List them.

5. List all vertices in the graph that are of distance 3 from (1,-2).

Day 6

RANDOM WALKS ON A PANCAKE GRAPH

A *path* in a graph is a listing of edges in a graph that can be traveled without picking up your pencil and without using any edge more than one time. As you look at your burnt pancake graph with two pancakes that you drew yesterday, you will see several paths from the vertex (1,-2) to the vertex (1,2). It is also possible to from one vertex to another without picking up your pencil but by possible reusing at least one edge. Such a listing of edges in the graph is called a *walk*. Note that every path is a walk, but not every walk is a path. You should be able to identify some walks in the burnt pancake graph that were not previously identified as paths. We now add one more component to the mix to more closely model the BPP for two pancakes. A bacterial cell does not know which two hix sites will be selected for flipping. Thus, even though we can see that it is possible to go from (1,-2) to (1,2) in a single flip, it would be unrealisitic to think that a bacterium (with no insight or foresight) would be flipped in the way that we can see would be most efficient. If the selection of which pair of hix sites is random, you would expect only 1/3 of the (1,-2) bacteria to reach (1,2) in a single flip. The following game is designed to help teach you about random walks in this burnt pancake graph. Investigation questions follow.

Random Walk Game: With 8 locations marked out on the floor of your room as shown above, label the locations as the vertices of the burnt pancake graph with two pancakes with (1,2), (1,-2), and so on. Note: While there is more than one way to lay out the labels, be sure that the labels are such that the implied edges accurately reflect the relationships in the graph. Remember that the connections in this graph represent the possibility to get from one stack to the other with a single flip. Now you will play a game in which you pretend you are a plasmid with the genes in the order you had them on day one (see Figure 2). Each person needs his or her own six-sided die and everyone starts by standing in the spot labeled (1, -2), which represents a promoter followed by a backwards coding region. Each person will roll their own die to simulate the random choice of a pair of hix sites by the Hin recombinase. If the die shows a 1 or a 2, move either east or west from your current position. If the die shows 3 or 4, move either north or south. If it shows 5 or 6, move from the inside square to the outside square (or vice versa) along the diagonal. Each person should keep track of the number of moves that he or she takes to reach the ending vertex labeled (1,2). Record these numbers on the board. Repeat game as necessary to collect a sufficient number of data points. Investigation questions:

1. What fraction of participants finished in 1 move? What fraction did you expect?

2. What fraction of participants finished in 2 moves? What fraction did you expect?

3. What fraction of participants finished in exactly 3 moves? What fraction did you expect?

4. What fraction of participants took more than 10 moves?

5. Based on your data, how many moves would you expect it to take to reach the finish?

6. Suppose the game did not finish when a participant gets to the (1,2) vertex but continues on with rolls of the dice and moves. What do you think would happen to the distribution of participants?

STUDY QUESTIONS

1. Synthetic biology projects have been conducted by undergraduate college students in the context of the International Genetically Engineered Machines competition, or iGEM, since 2005. Find and describe an example of an iGEM project in each of the categories medicine, energy, and the environment.

2. What does the term "computer" mean? Describe what you think are the requirements for a device to be called a computer. Is it possible for a bacterial cell or a population of bacteria to function as a computer?

3. Describe the purpose of genetic recombination in nature. For what purpose do *Salmonella* bacteria use the Hin/hix recombination system?

4. Construct a pancake problem of your own that involves five burnt pancakes. Have a friend try to solve the problem. Was your problem relatively hard or easy?

5. The pancake problem you investigated involved a promoter and a gene. How could a larger burnt pancake problem be encoded in bacteria?