Solving the Pancake Problem with a Bacterial Computer

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Our Collaboration

Davidson College

3 Biology students
1 Math student
1 post-doc
2 faculty in Biology and Math

Missouri Western State University

5 Biology students
1 Math student
1 High School student
2 faculty in Biology and Math

Goals

- Have fun with synthetic biology
- Integrate math and biology
- Test iGEM collaboration among PUIs
- Solve a math problem using synthetic biology
- Design a device with downstream apps
- Have more fun with synthetic biology

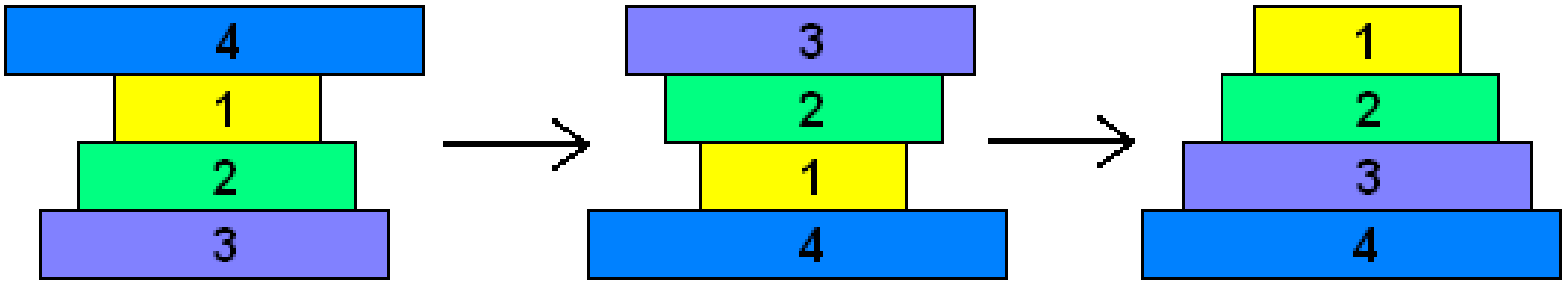
The Classic Pancake Problem

- Scenario
  - Pancake chef at iHOP
  - Spatula in one hand
  - Plate with a stack of delicious pancakes of different sizes in other hand
  - No place to set down the plate
- Problem
  - The chef wishes to serve the pancakes arranged from smallest to largest
  - How many flips are needed?

A Simple Model

Given a particular permutation, we want to find the least number of flips needed to obtain the arrangement 1,2,3,4.

In our example, we consider 4,1,2,3.

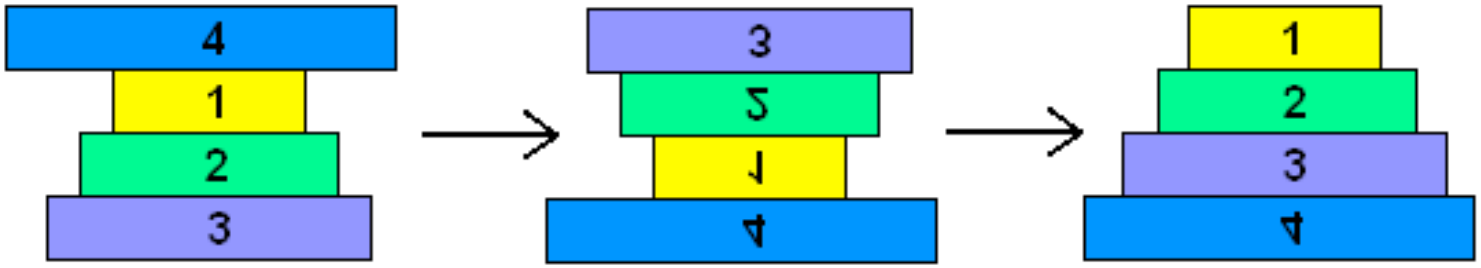


The Burnt Pancake Problem

- Modification of the Classic Pancake Problem
  - Each pancake has one burnt side
- Problem
  - Sort pancakes from smallest to largest, all burnt-side down
  - How many flips are needed?

A Burnt Pancake Model

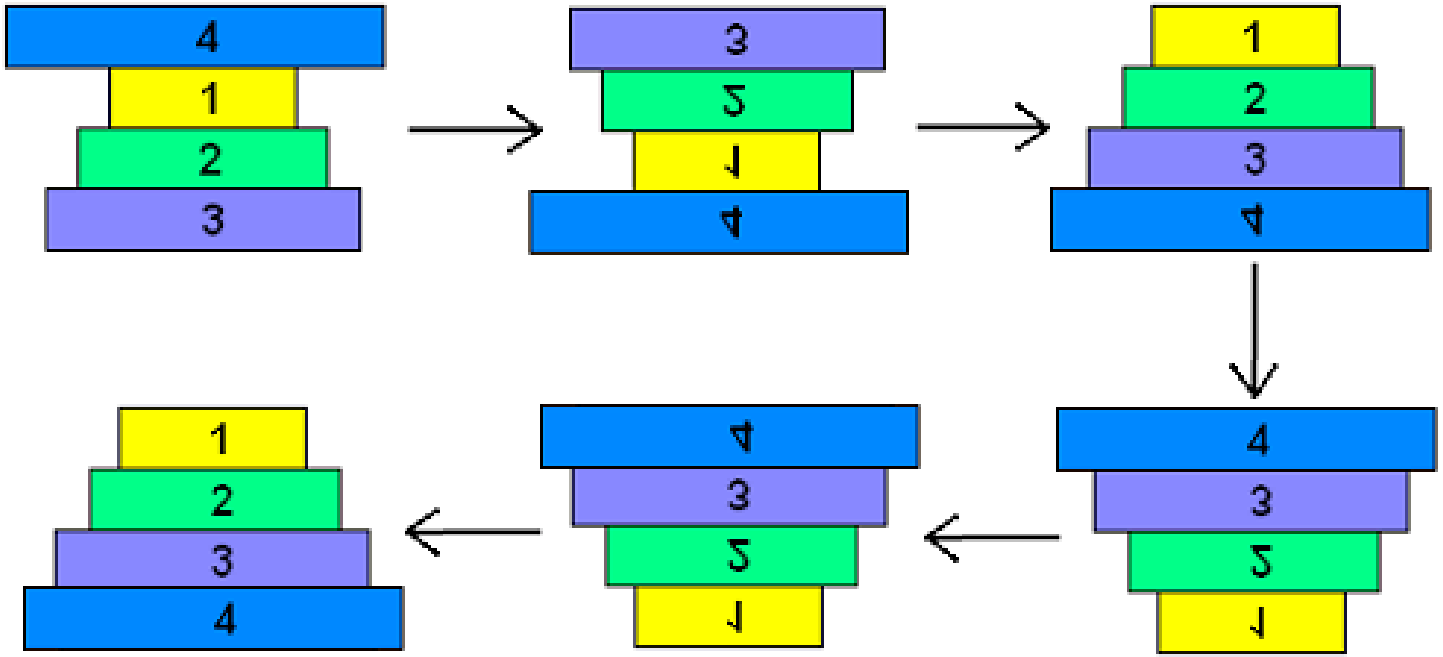
Same stack as earlier, same flips.



The bottom pancake is upside down so more flips are needed for the burnt pancake problem.

A Burnt Pancake Model (cont.)

We could continue from where we left off.

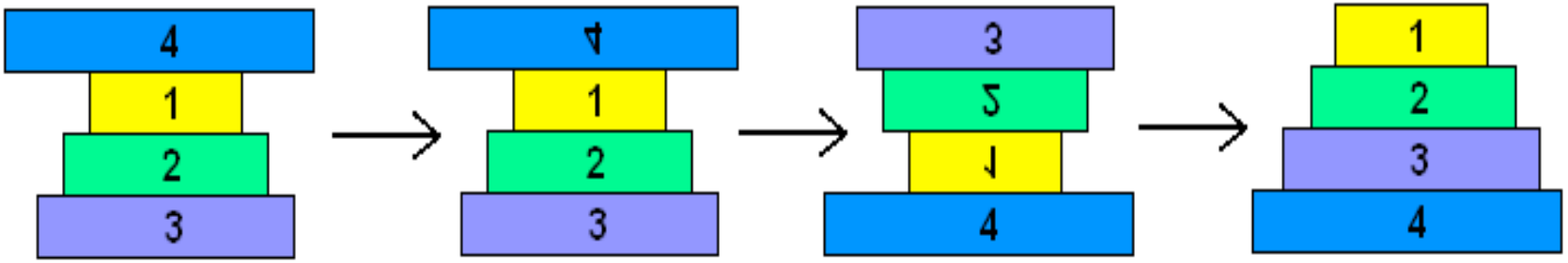


There could be a more efficient way.

A Burnt Pancake Model (cont.)

We want the most efficient way to change the stack.

Below we use only three flips instead of five.



Hin-Hix Recombination

- *Salmonella* uses recombination to achieve antigenic variation
- Target DNA contains promoter that drives either of 2 flagellar protein genes

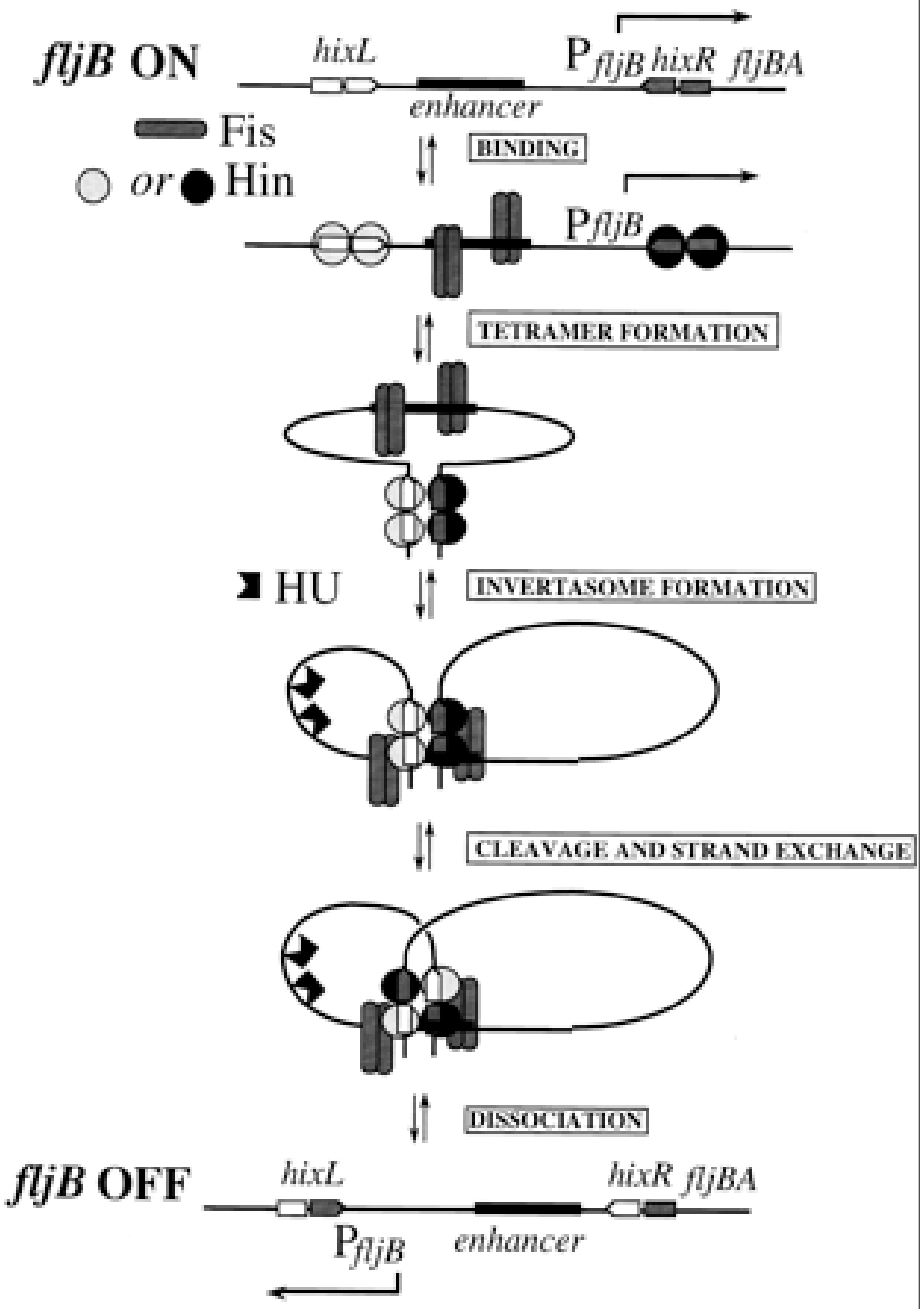


Image from: Nanassy OZ and Hughe K, 1998 *Genetics* 149: 1649-1663.

Recombination Requirements

- *Cis* Elements
  - hixL and hixR bracket target
  - Recombination enhancer
  - Negative supercoiling
- *Trans* Elements
  - Hin recombinase
  - Fis (factor for inversion stimulation)
  - HU (heat-unstable nucleoid protein)

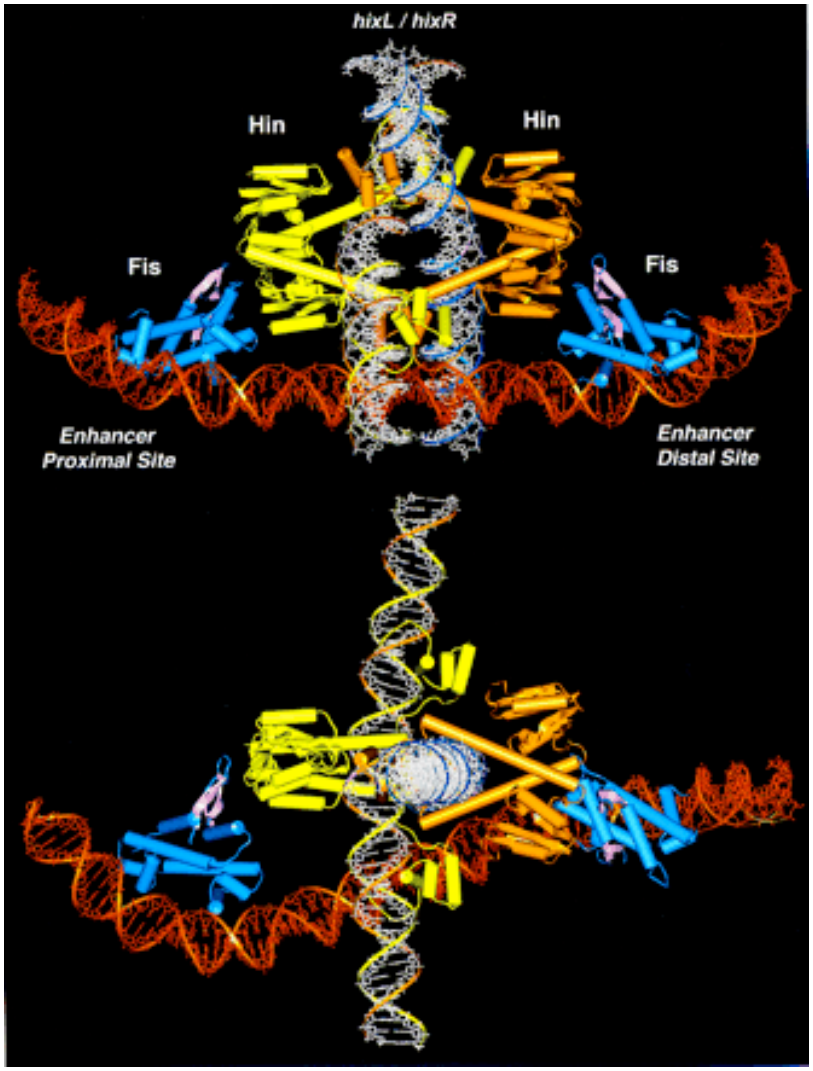


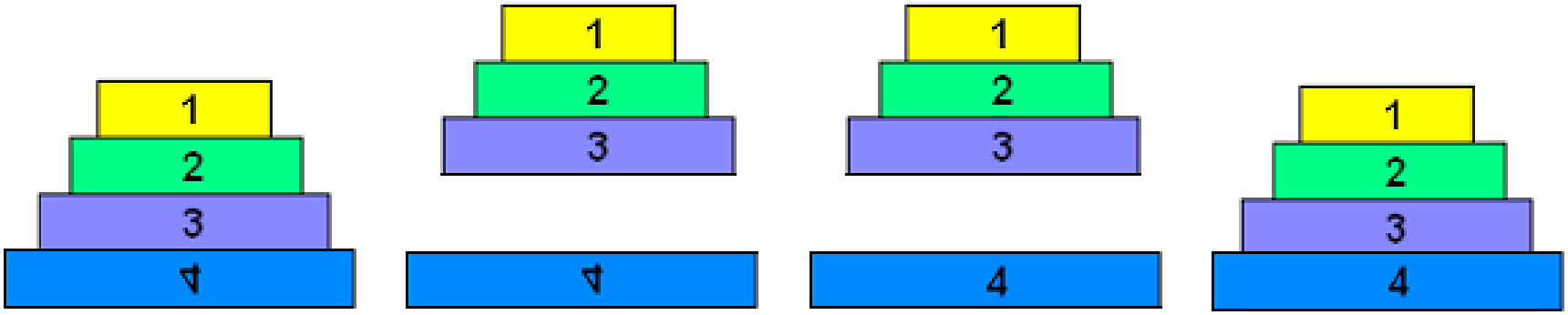
Image from: Merickel SK, Haykinson MJ, and Johnson RC, 1998. *Genes Devel* 12, 2803-2816.

Burnt Pancake Implementation

- Use Hin recombinase system to generate the solution to the burnt pancake problem
- Types of burnt pancakes
  - Promoter
  - RBS / coding sequence
  - terminator
- Needed for flipping
  - Hin recombinase inducible expression cassette
  - HixC sites bracketing each pancake
  - RE may be needed
- Detection of flipping
  - Genetic detection using inducible antibiotic resistance or color

Which DNA Pancake Problem Can We Solve?

- Middle pancakes can be flipped, not just top
- This is modification of the burnt pancake problem
- A chef with two spatulas
  - lift top of stack
  - flip top portion of remaining stack
  - replace top of stack without flipping



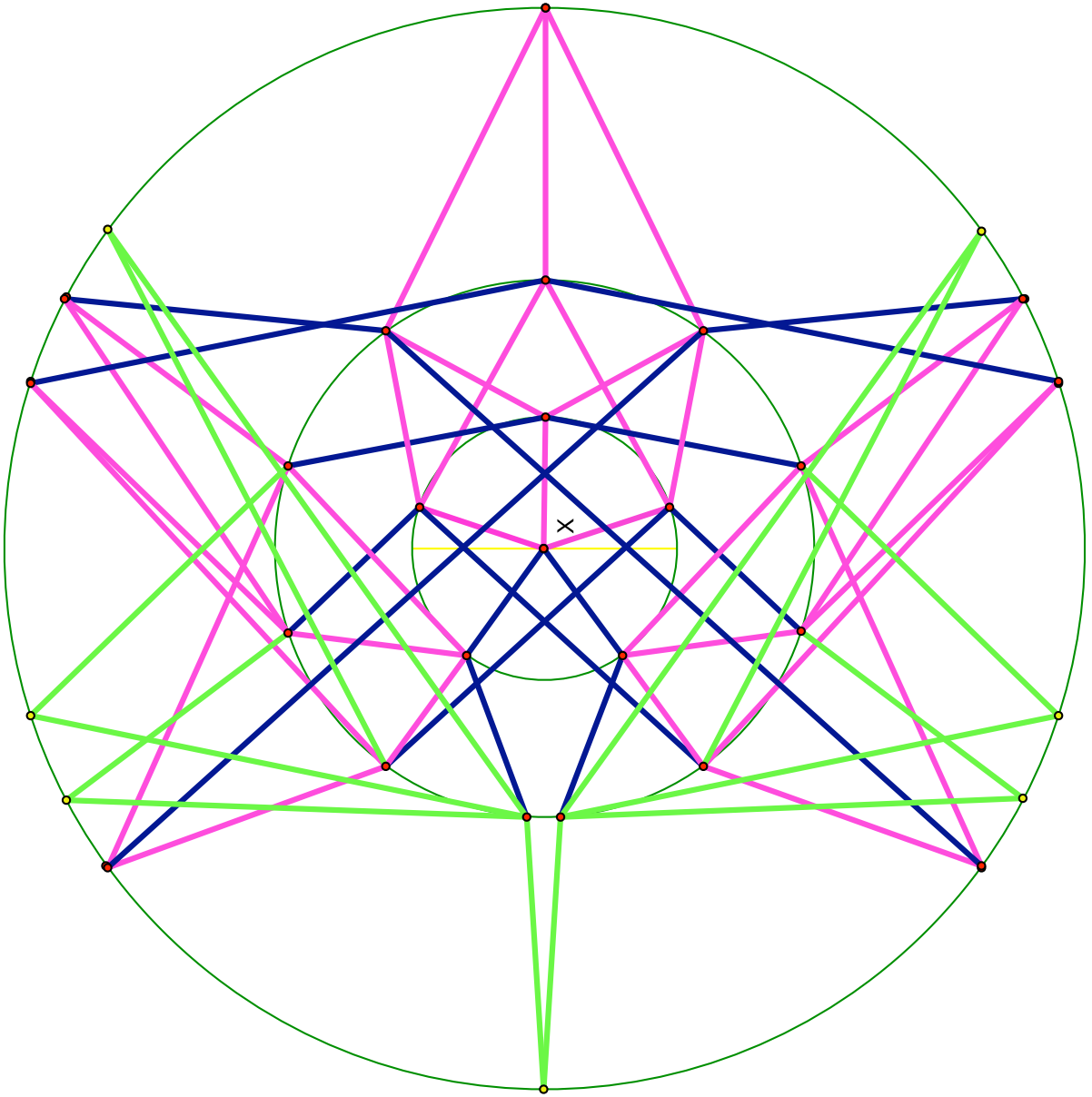
2-Spatula Burnt Pancake Graph for 3 pancakes

- 3 pancakes $\longrightarrow$ 48 possible stacks
- Each stack is one flip away from six others

  first only, second only, third only,

  first and second, second and third, all three.
- The following slide shows Northern Hemisphere of the 2-spatula burnt pancake graph on a globe.
- Each stack is diametrically opposite the stack related by flipping all three pancakes.

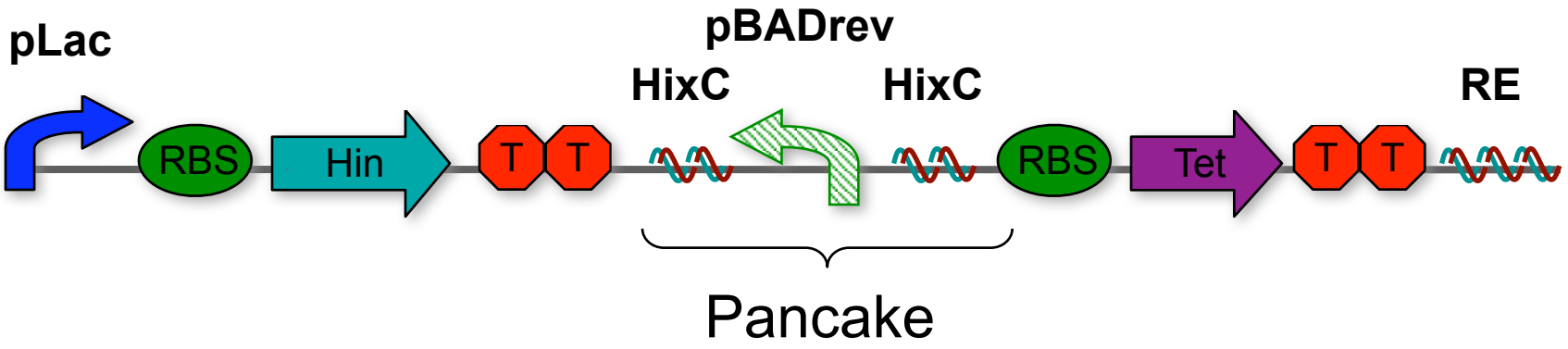
Northern Hemisphere



Experimental Goals

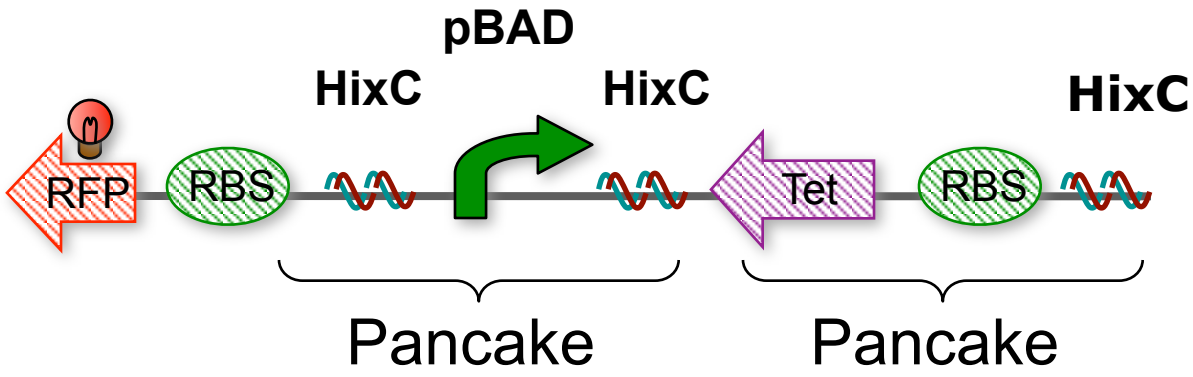
- Design a system in *E. coli* to test whether flipping occurs
  - Single pancake constructs
  - Result of flipping is gene expression
- Determine whether flipping of multiple pancakes can occur
  - Two pancake constructs
  - Four pancake constructs
- Measure pathways and kinetics of flipping

A One Pancake Construct



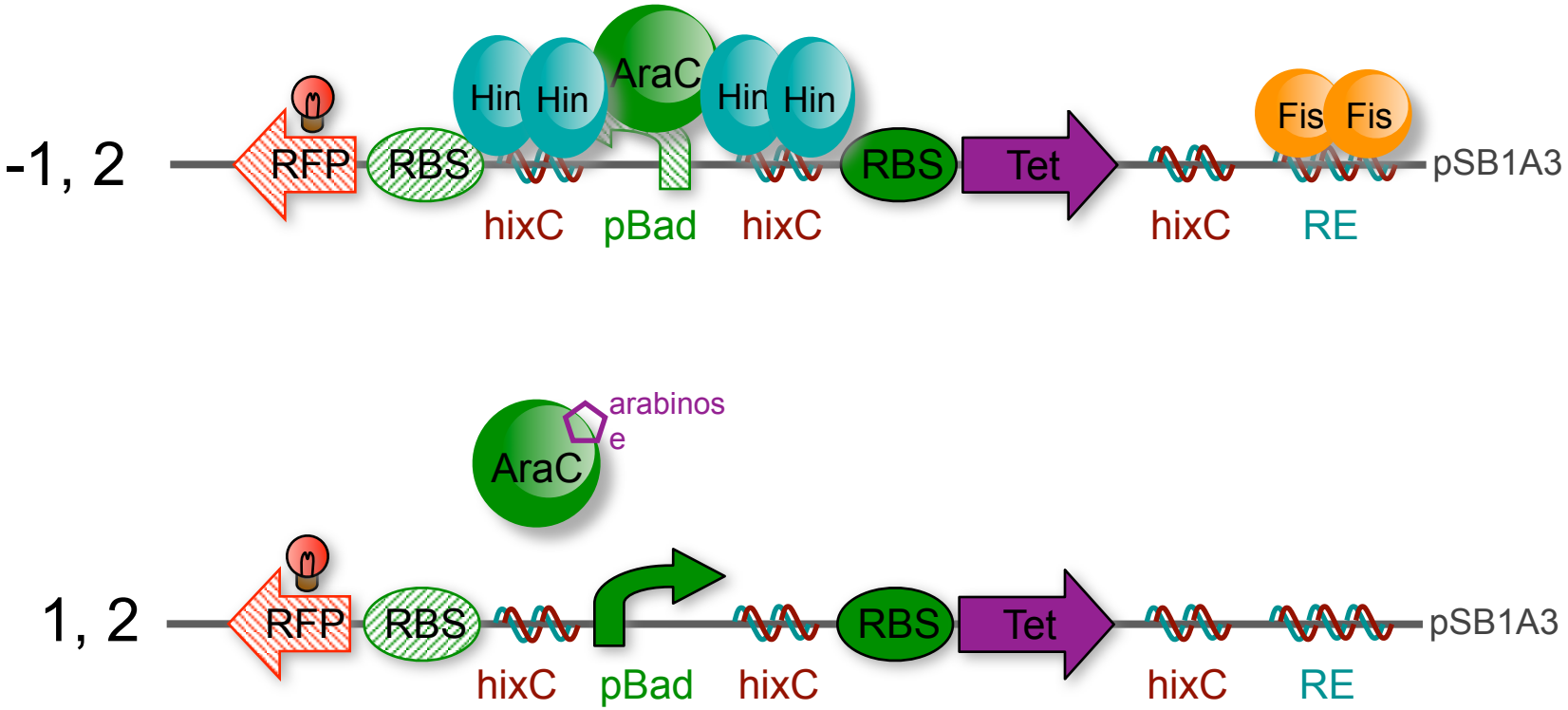
- Hin expression under control of pLac
- Starting configuration is Tet sensitive
- Flipping of pBADrev pancake results in Tet resistance

A Two Pancake Construct



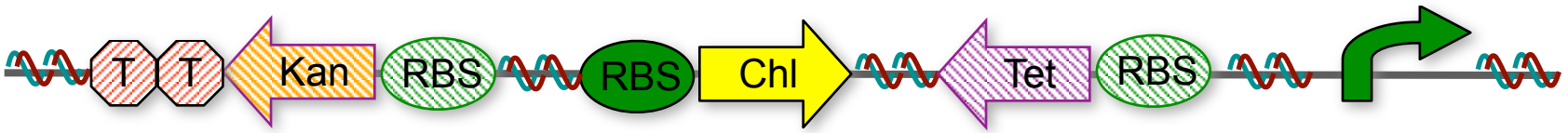
- Hin provided by separate plasmid
- Starting configuration is Tet sensitive
- Flipping results in 8 different configurations,
  - 1 is Tet resistant
  - at least 4 have RFP expression

Proteins Interacting With 2-Pancake Construct

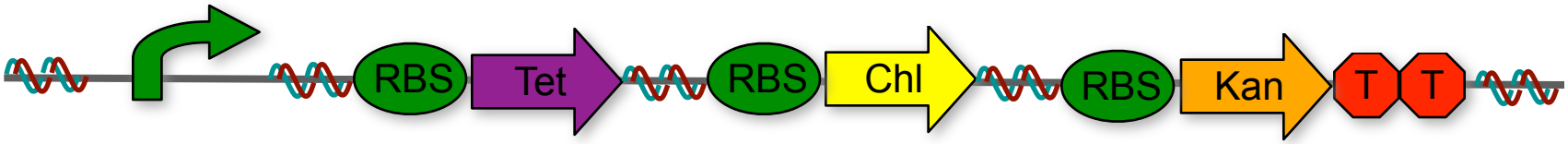


Four Pancake Constructs

- Starting configuration is Tet, Chl, Kan sensitive

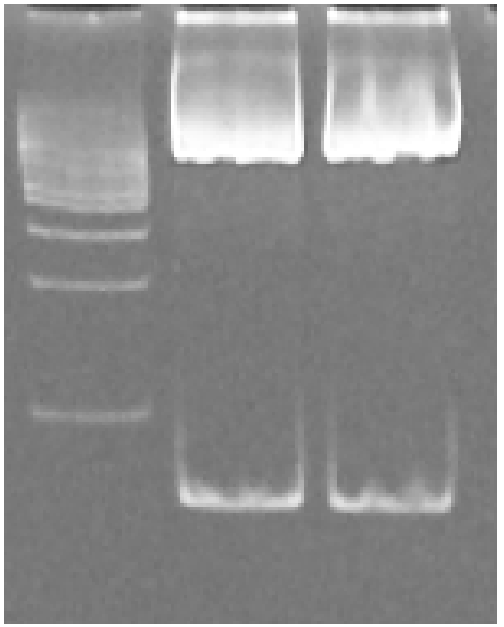


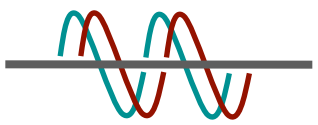
- Flipping results in various configurations of Tet^R, Chl^R, Kan^R.



Methods – Building New Parts

- Synthetic DNA
  - Determine sequence
  - Order DNA to be made
  - Anneal oligos
  - Ligate into pSB1A2
  - Verify with sequencing
- Method used to make:
  - hixC
  - Recombination Enhancer (RE)
  - Reverse RBS

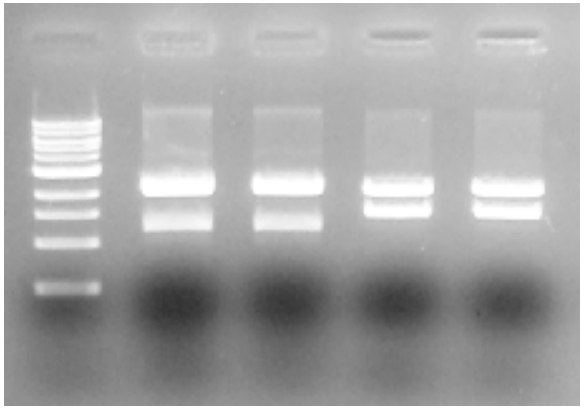


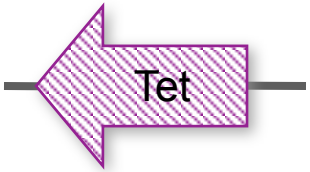


hixC

Methods – PCR of Natural Genes

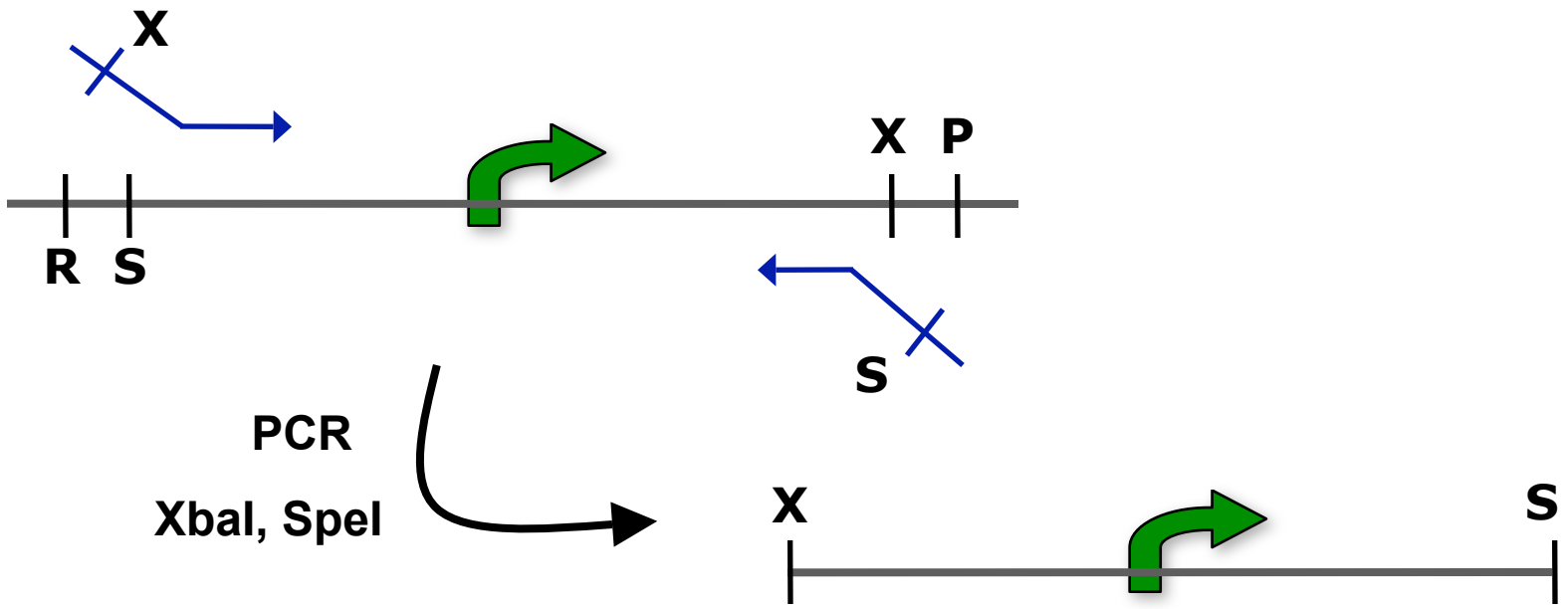
- Amplification
  - Locate gene and design primers
  - Isolate genomic DNA
  - Optimize PCR reaction
  - Purify band
  - Clone into pSB1A2
- Method used to make:
  - Hin recombinase from *Salmonella*
  - Hin recombinase with LVA tag
  - 3 antibiotic resistance genes from *E. coli*





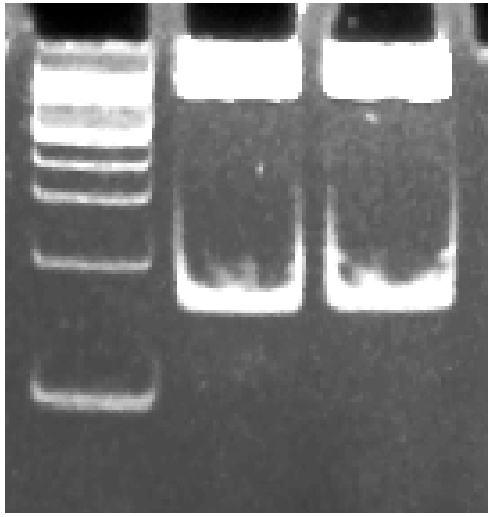
Methods – Reversal of Parts

- PCR Switcharoo
  - Primers with BB prefix and suffix switched but also complementary to part
  - Purify XbaI/SpeI fragment and clone into pSB1A2



Methods – Reversal of Parts

- Method used to make
  - Reversed pBAD
  - 3 Reversed antibiotic resistance genes



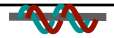
pBADrev

Parts Contributed to the Registry

- Basic Parts
  - 14 basics parts (11 + 3)
    - Recombination parts
    - New cloning vector
    - New Ab resistance genes forward and reverse
    - New control elements
- Construction Intermediates
  - 25 contributed (18 + 7)
- Devices
  - 23 contributed (12 + 11)

Color Key: Red=Davidson Blue=Missouri Western

Basic Pancake Parts Contributed

Name	Icon	Description
J31009		pSB1A7 (insulated plasmid)
J31000		Hin Recombinase
J31001		Hin Recombinase-LVA
J3101		Recombination Enhancer
J44000		HixC
J44001		RBS reverse
J31003		Kan^R forward
J31002		Kan^R reverse
J31005		Chl^R forward
J31004		Chl^R reverse
J31007		Tet^R forward
J31006		Tet^R reverse
J44002		pBAD reverse
J31011		RFP and RBS reverse

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Solving the Pancake Problem with a Bacterial Computer

Thanks to the Missouri Western Summer Research Institute and Student Excellence Fund, to the iGEM Founders, Organizers, and Community, to *His Ambassadorship,* Andrew Hessel, and to our collaborators at Davidson College

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