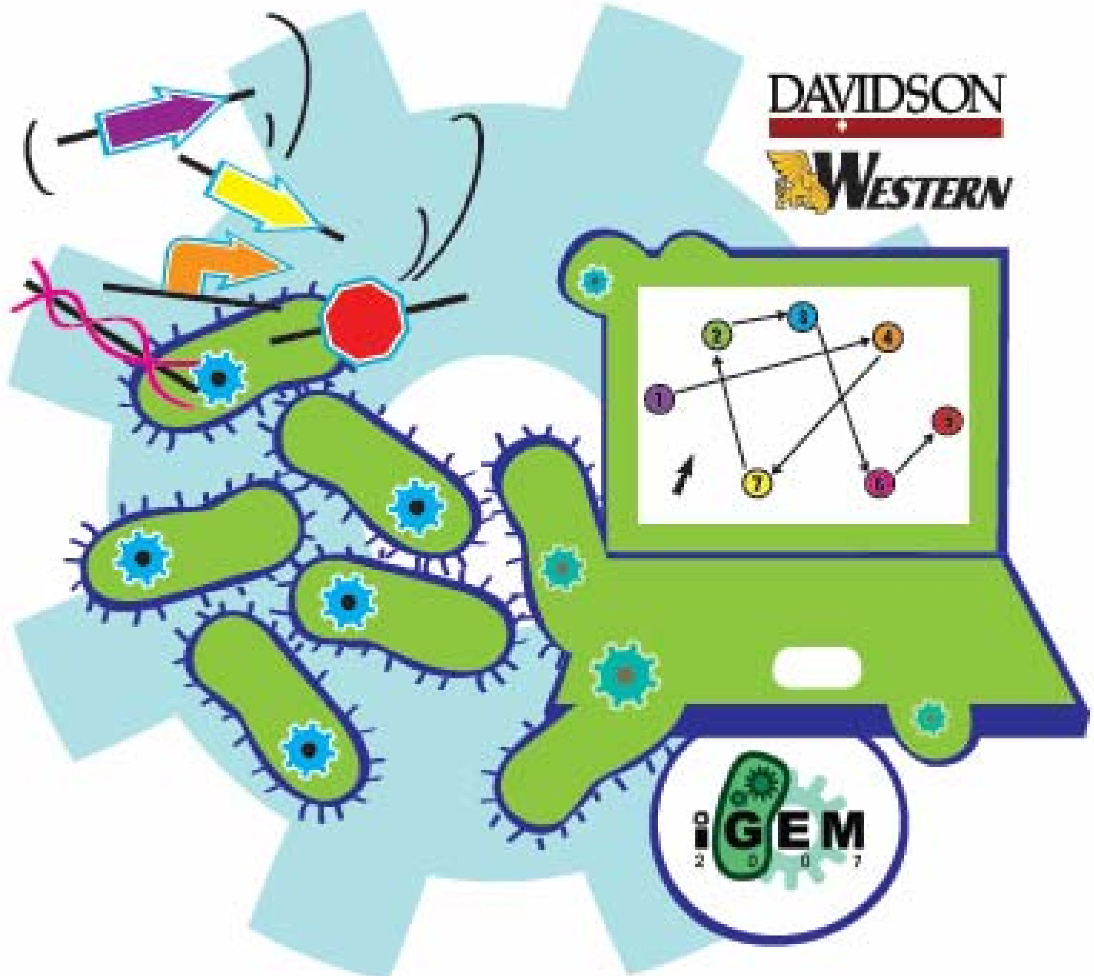
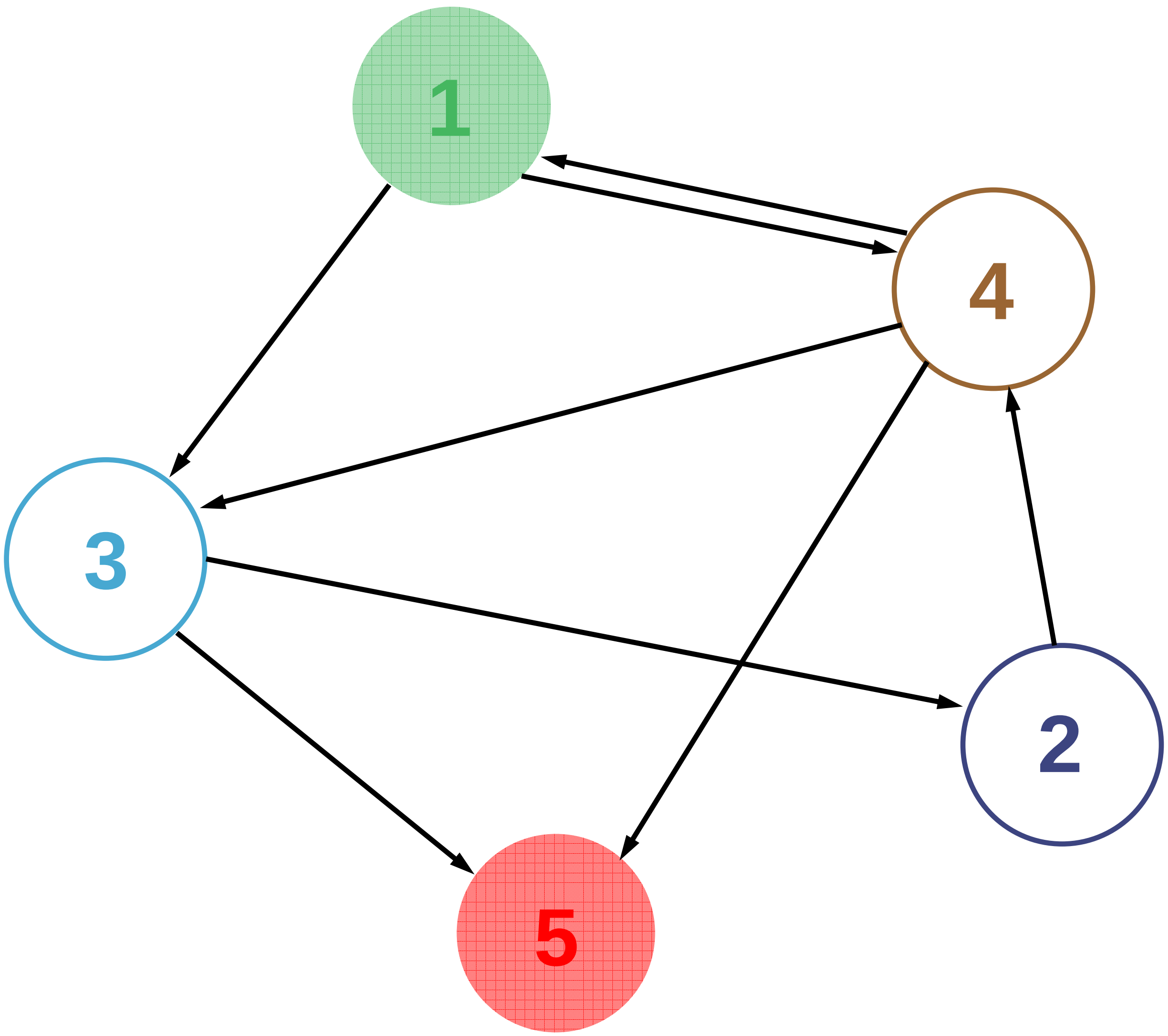
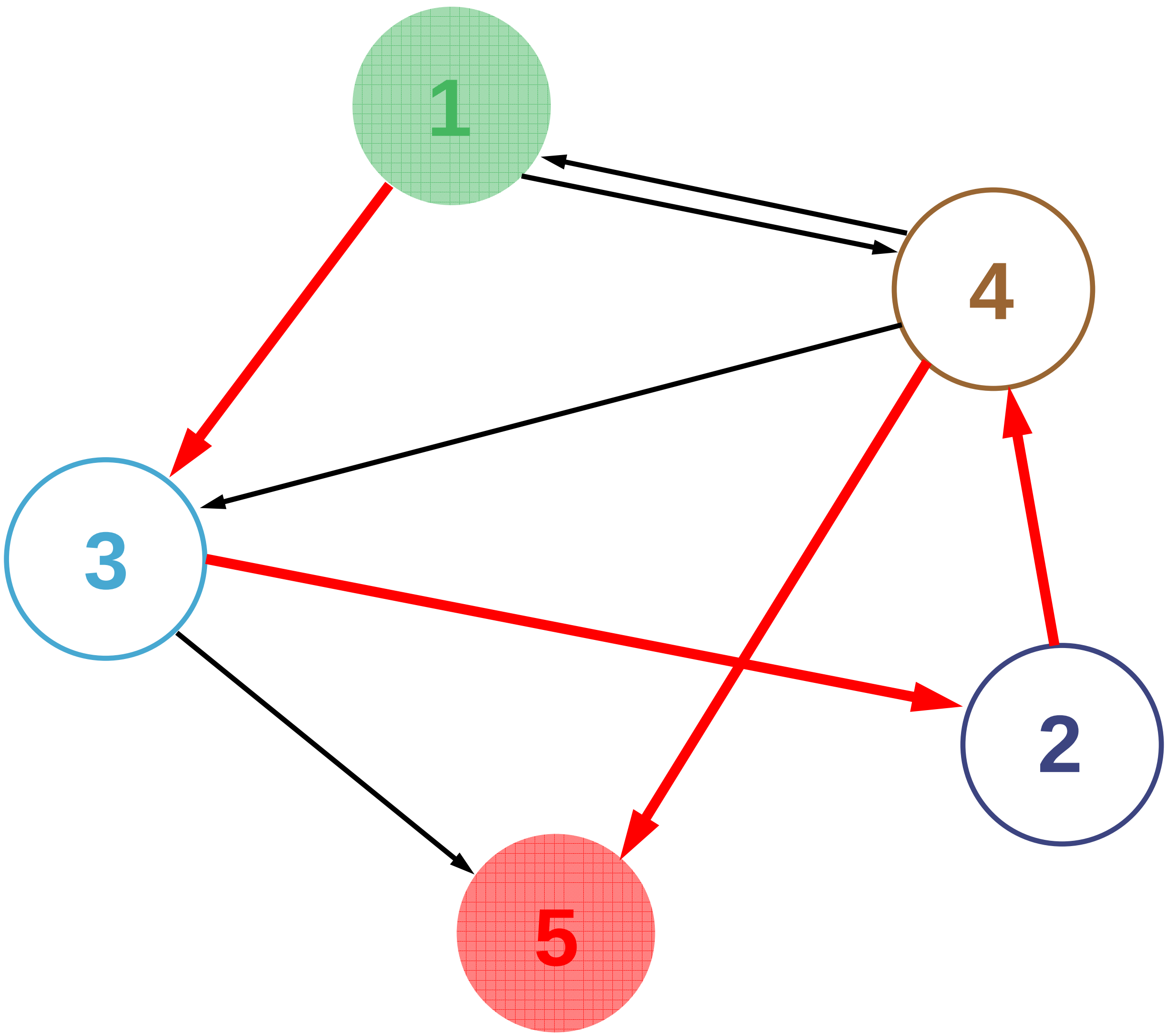
Solving the Hamiltonian Path Problem with Living Hardware



The Hamiltonian Path Problem



The Hamiltonian Path Problem



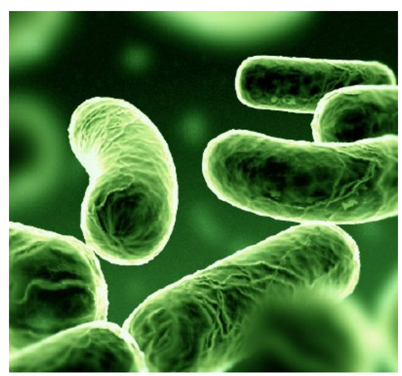
Advantages of Bacterial Computation

Software → Hardware → Computation

 →  → Computation

http://www.dnamnd.med.usyd.edu.au/

→



http://www.turbosquid.com

→ Computation

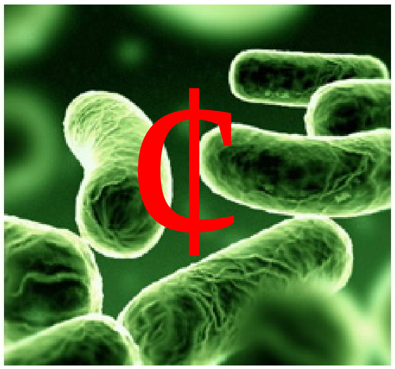
Advantages of Bacterial Computation

Software → Hardware → Computation

 → 

→ Computation

http://www.dnamnd.med.usyd.edu.au/

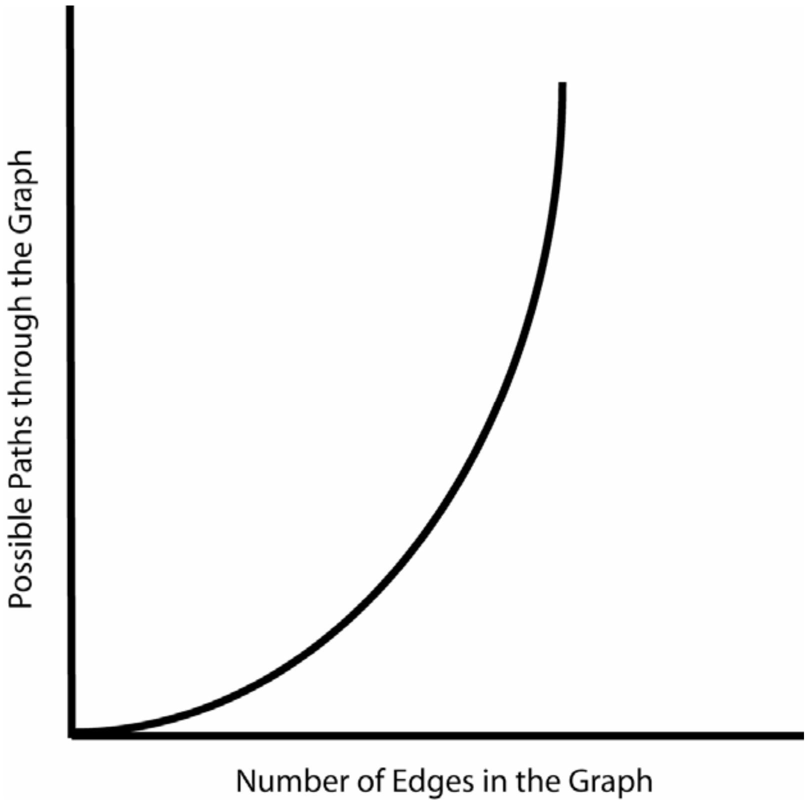
→ 

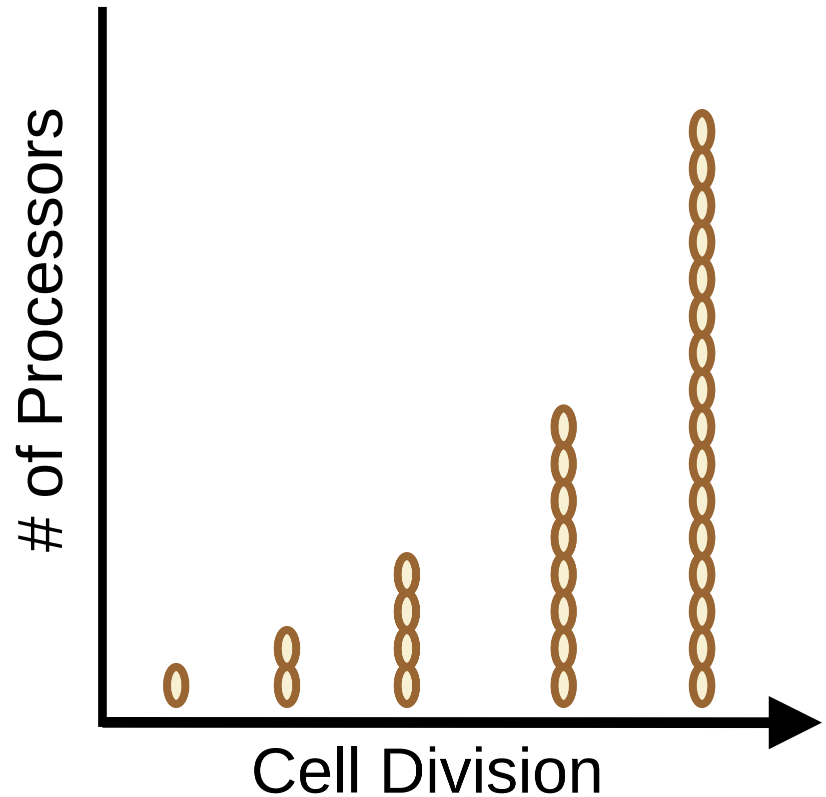
http://www.turbosquid.com

→ Computation

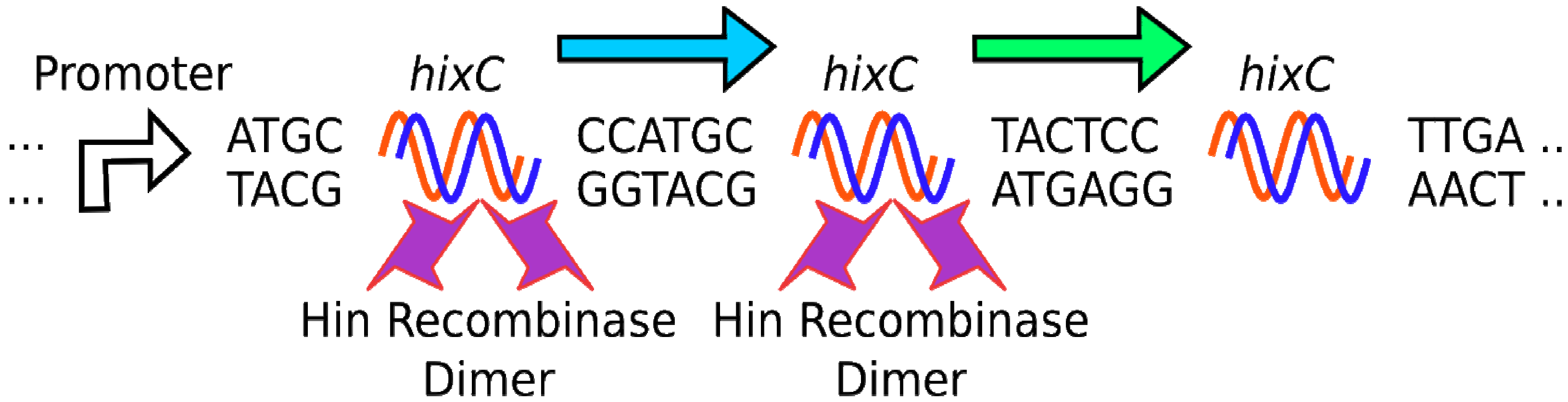
Computational Complexity

- Non-Polynomial (NP)
- No Efficient Algorithms





Flipping DNA with Hin/*hixC*



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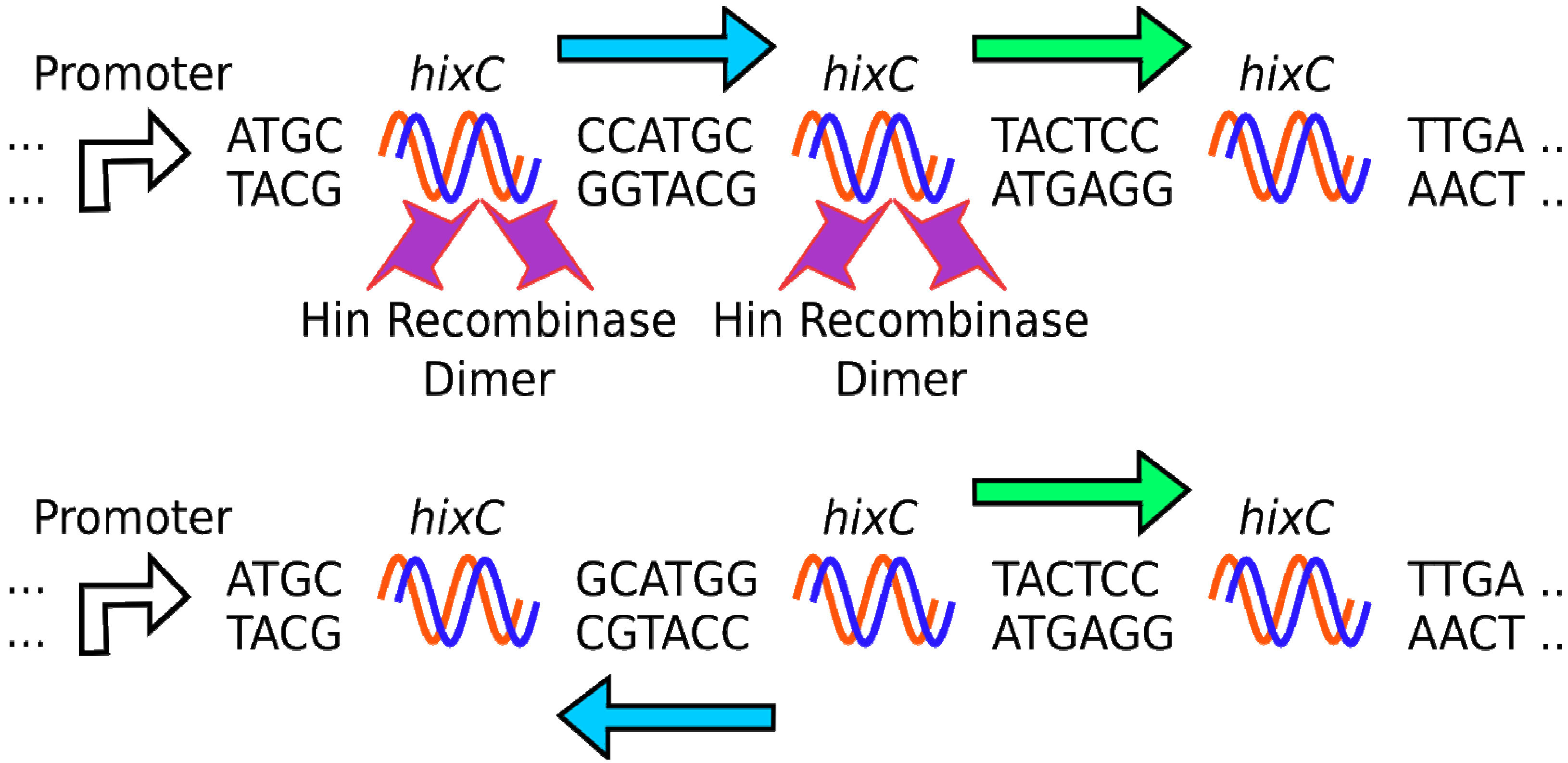
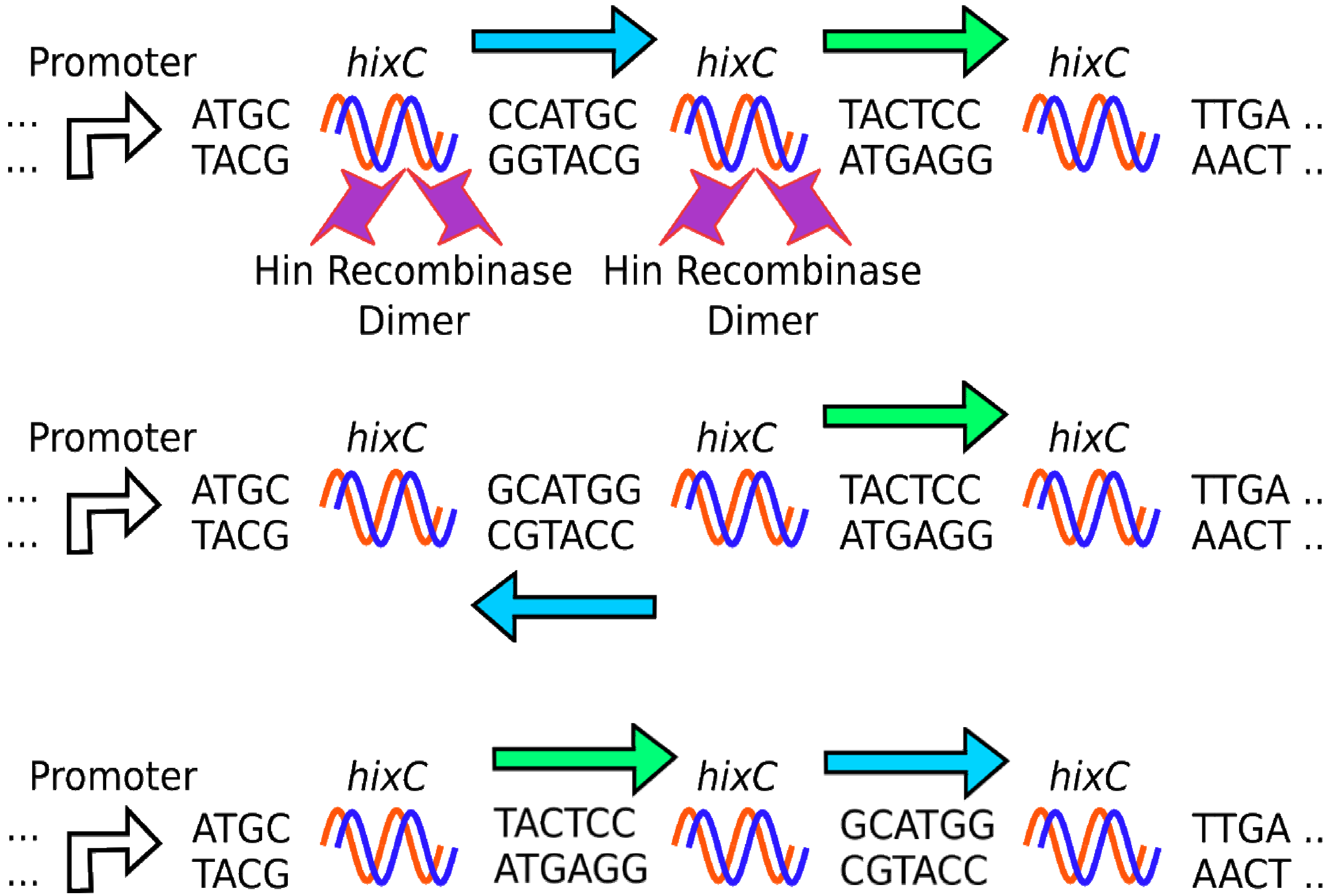
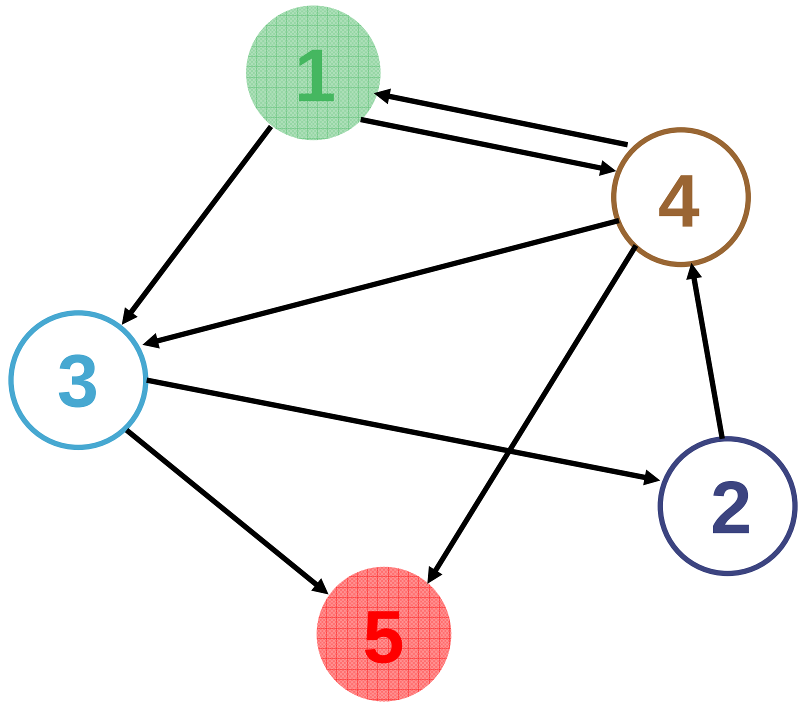
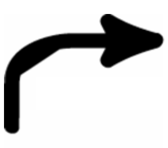


Flipping DNA with Hin/*hixC*



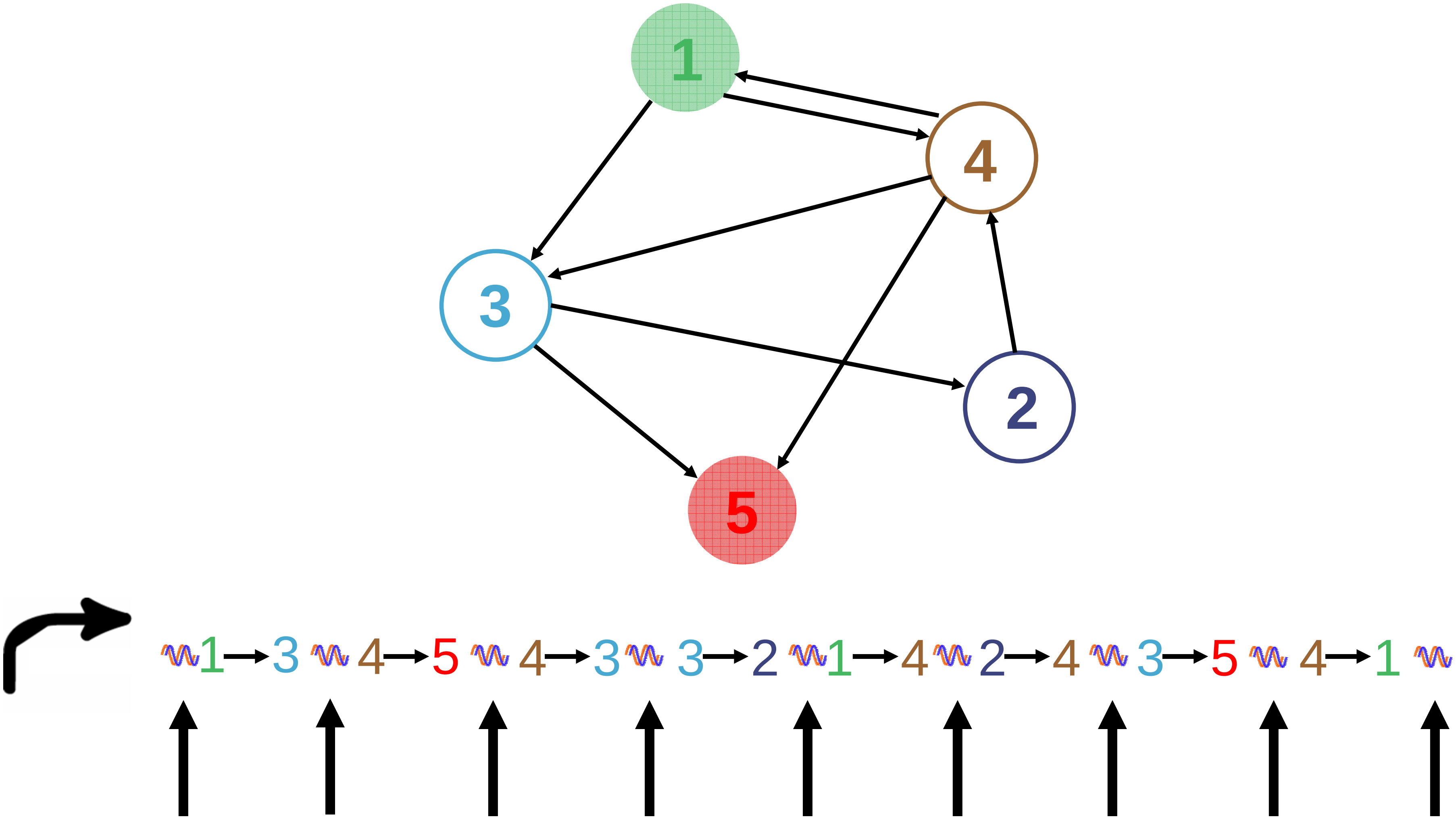
Encoding a Graph into DNA





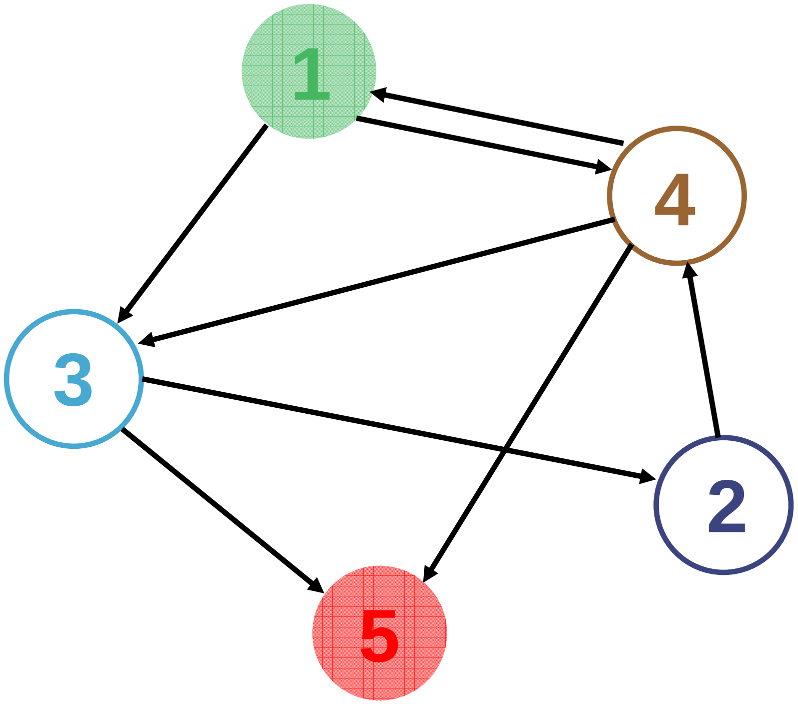
1→3 4→5 4→3 3→2 1→4 2→4 3→5 4→1

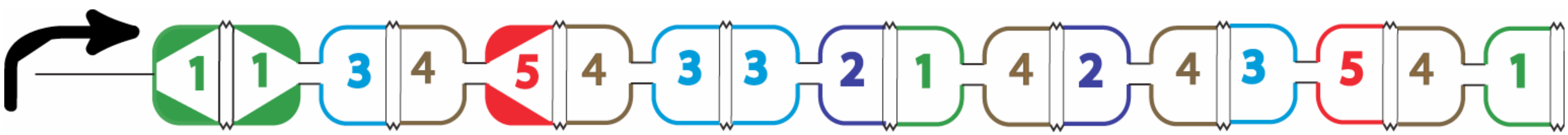
Encoding a Graph into DNA



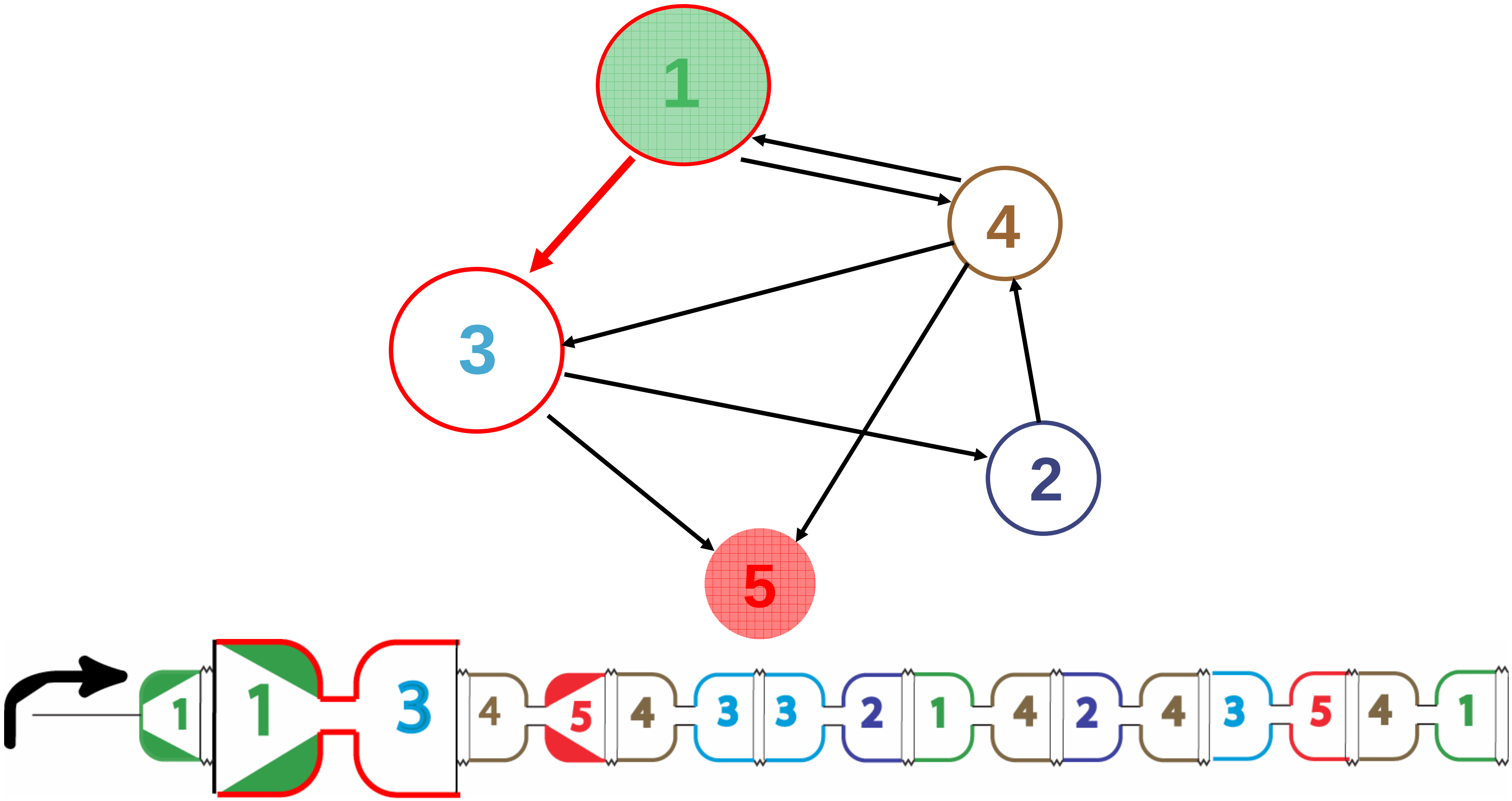
hixC Sites

Encoding a Graph into DNA

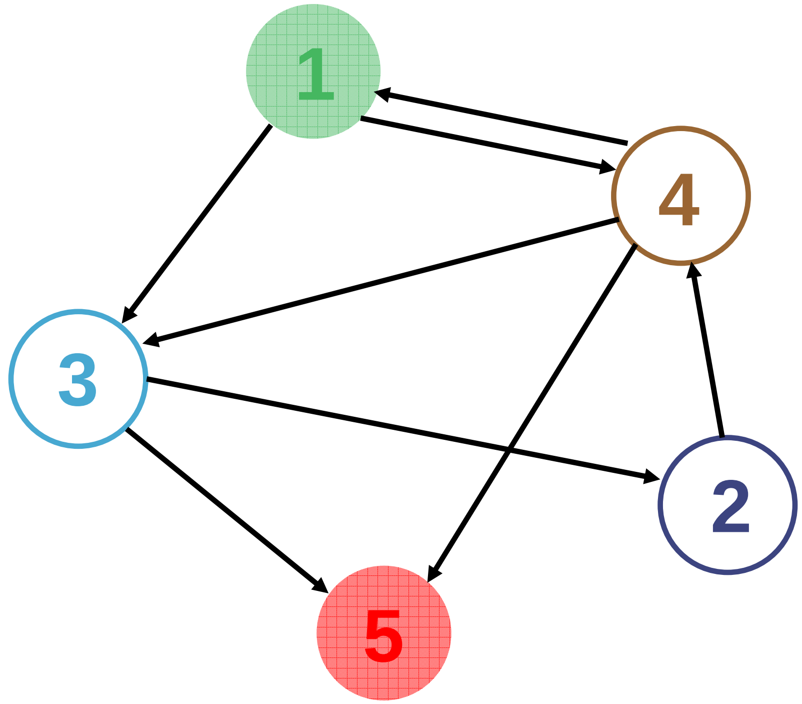


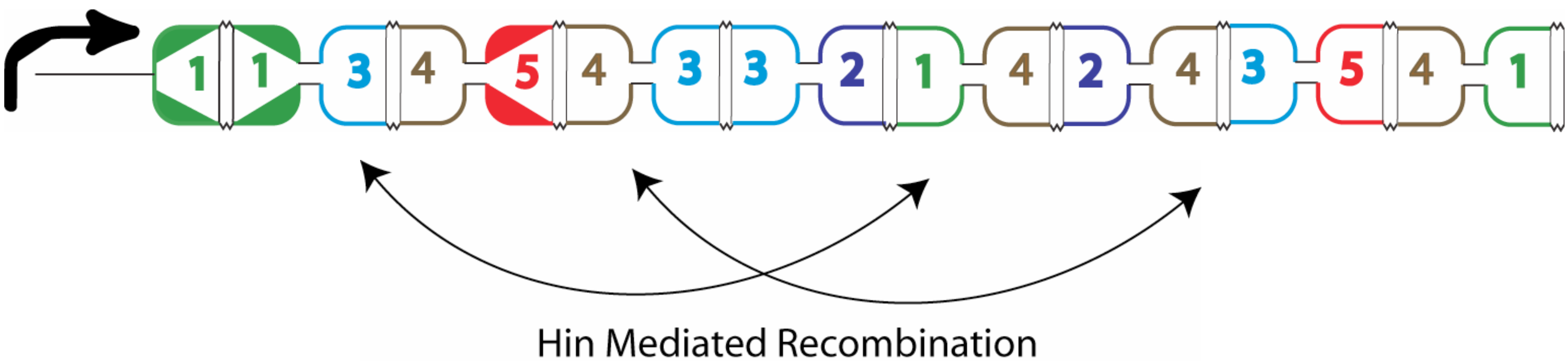


Encoding a Graph into DNA

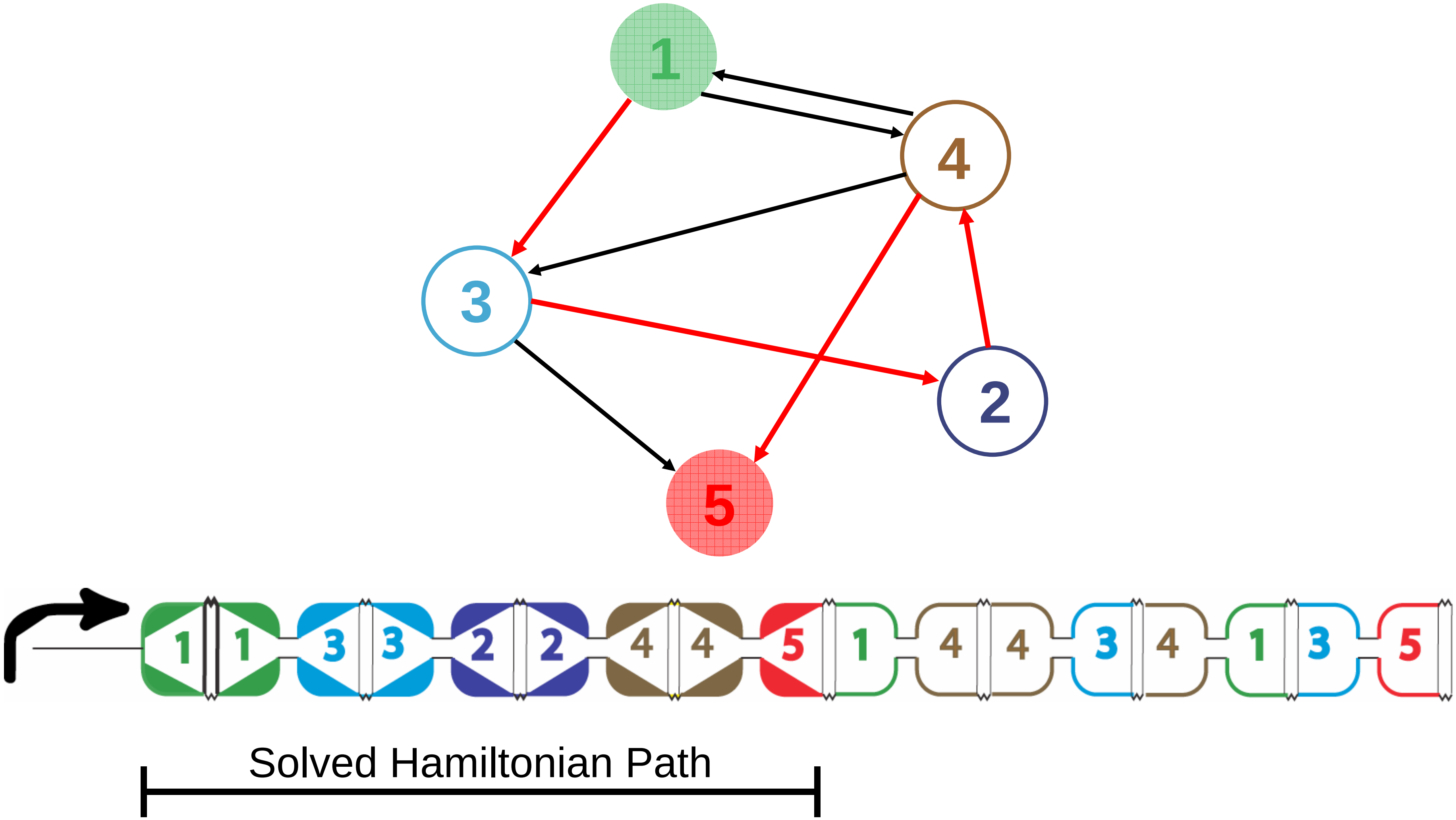


Using Hin/*hixC* to Solve the HPP





Using Hin/*hixC* to Solve the HPP



Solved Hamiltonian Path

Predicting Outcomes of Bacterial Computation

How Many Plasmids Do We Need?

Probability of at least *k* solutions on *m* plasmids for a 14-edge graph

	k = 1	5	10	20
m = 10,000,000	.0697	0	0	0
50,000,000	.3032	.00004	0	0
100,000,000	.5145	.0009	0	0
200,000,000	.7643	.0161	.000003	0
500,000,000	.973	.2961	.0041	0
1,000,000,000	**.9992**	.8466	.1932	.00007

k = actual number of occurrences

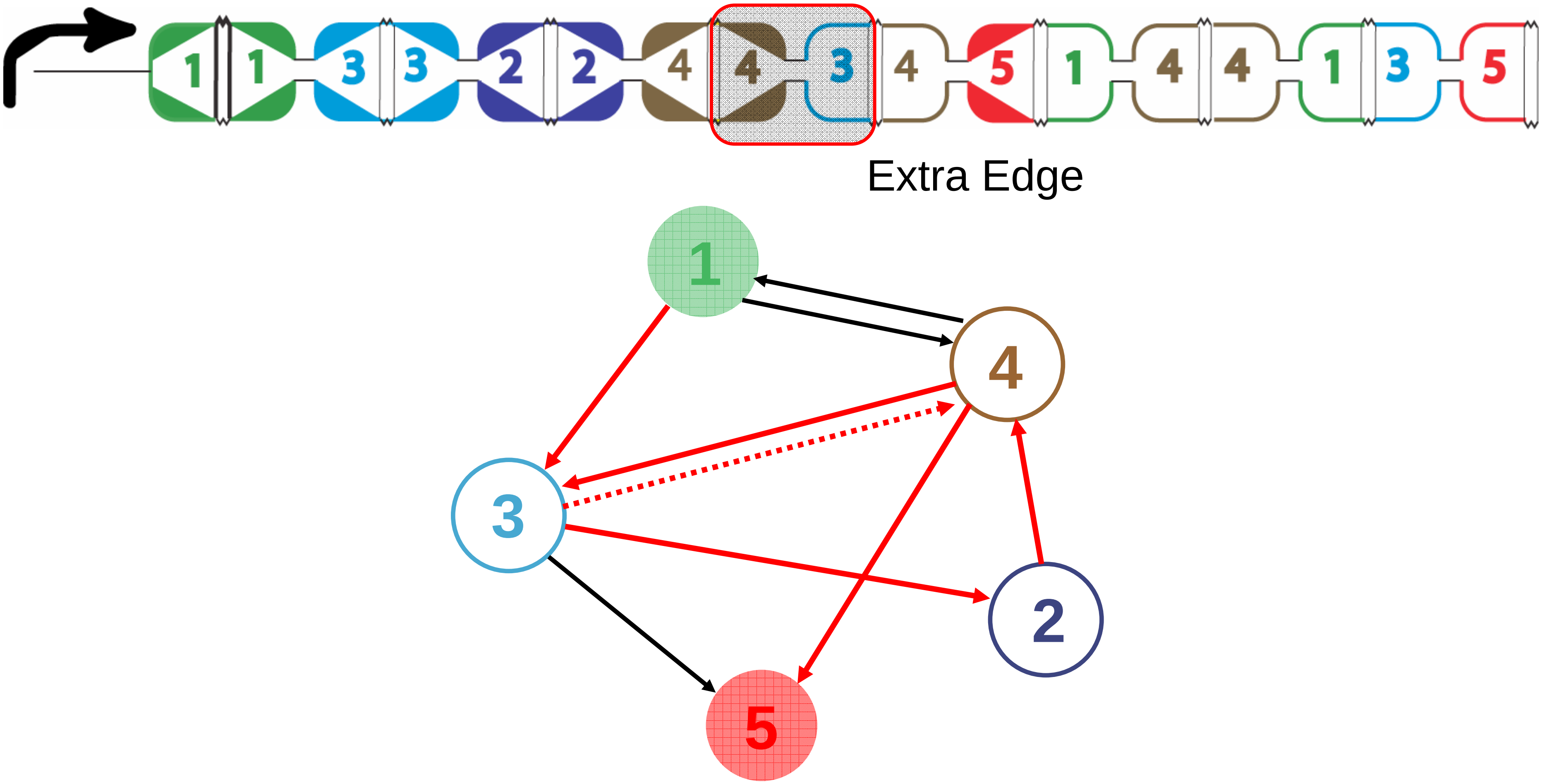
λ = expected number of occurrences

λ = *m* plasmids * # solved permutations of edges ÷ # permutations of edges

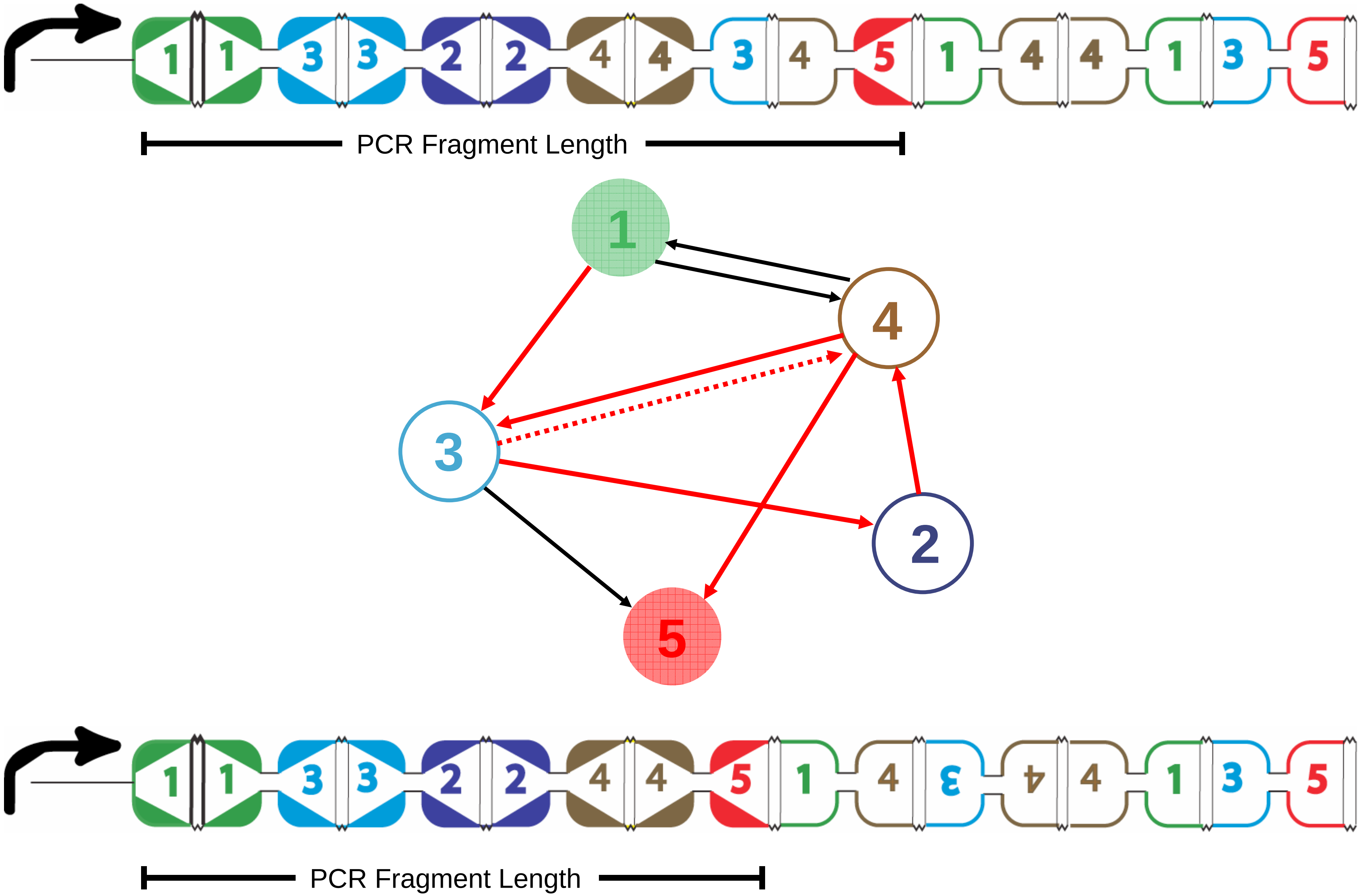
Cumulative Poisson Distribution:

$$P(\text{\# of solutions} \geq k) = 1 - \sum_{x=0}^{k-1} \frac{e^{-\lambda} \bullet \lambda^{x}}{x!}$$

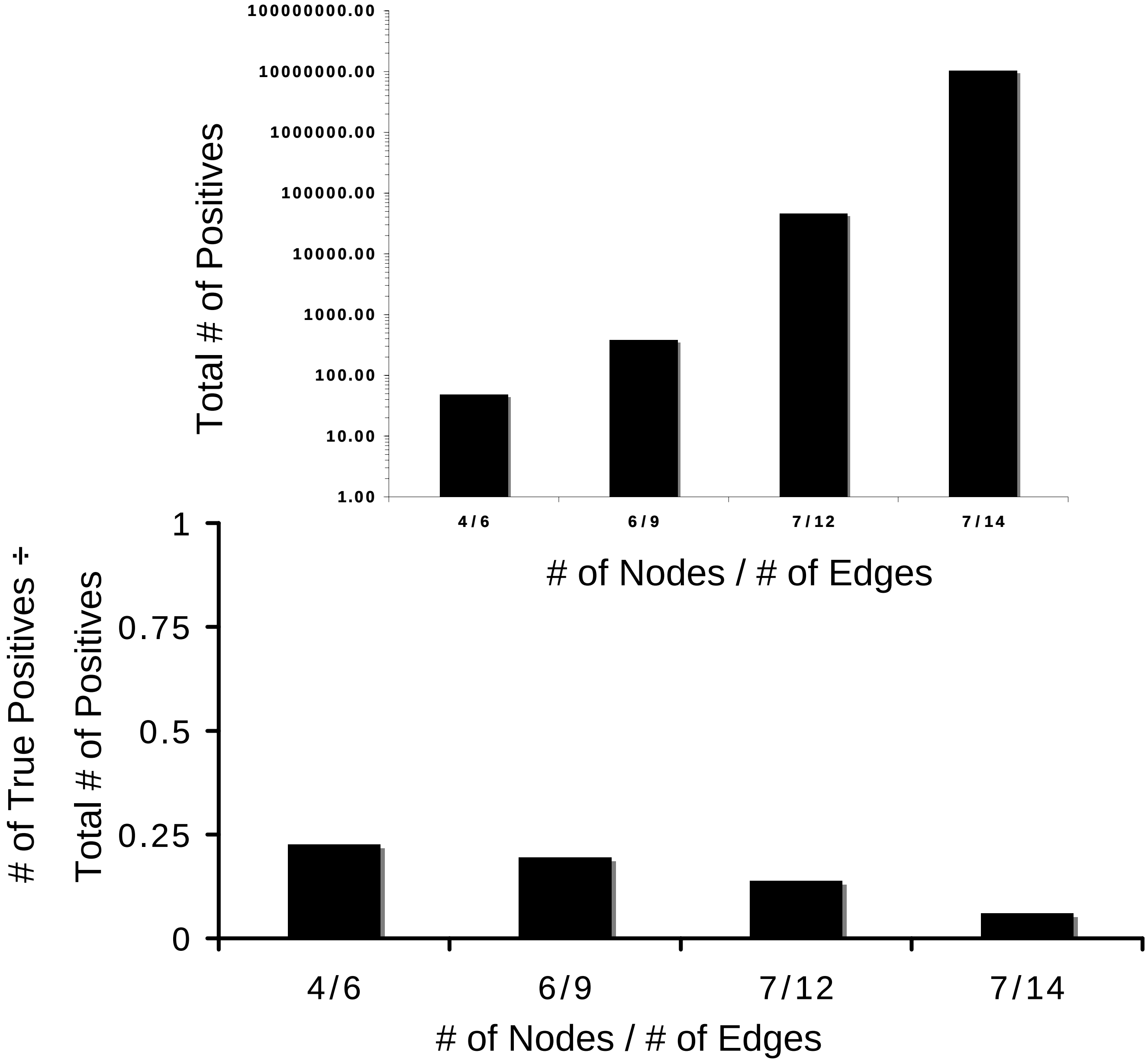
False Positives



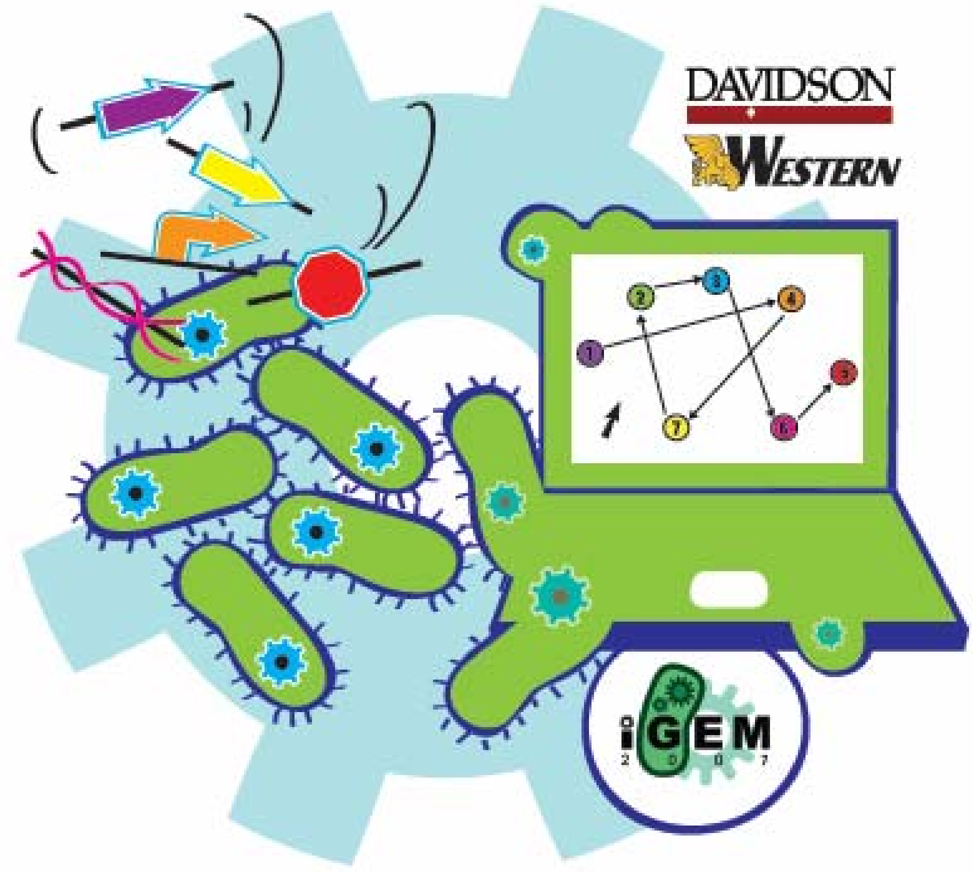
False Positives



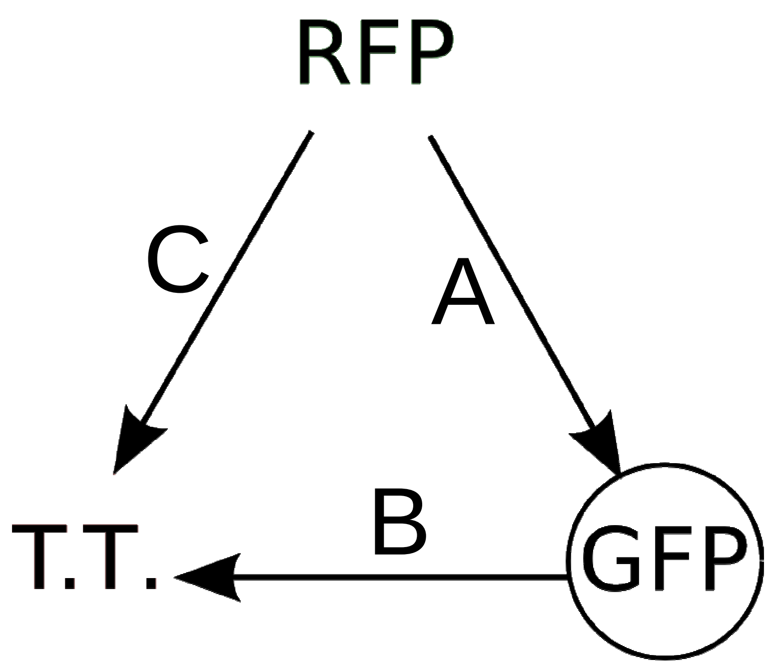
Detection of True Positives



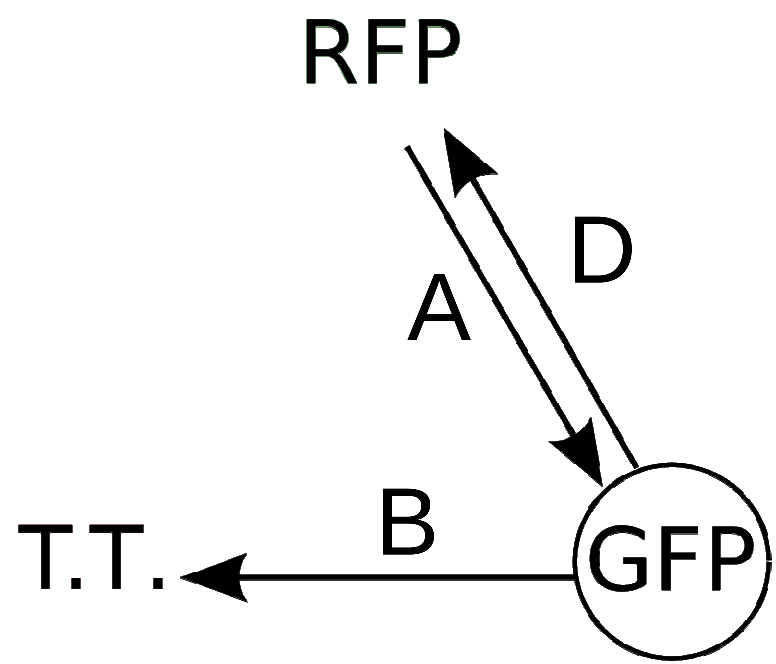
Building a Bacterial Computer



Choosing Graphs

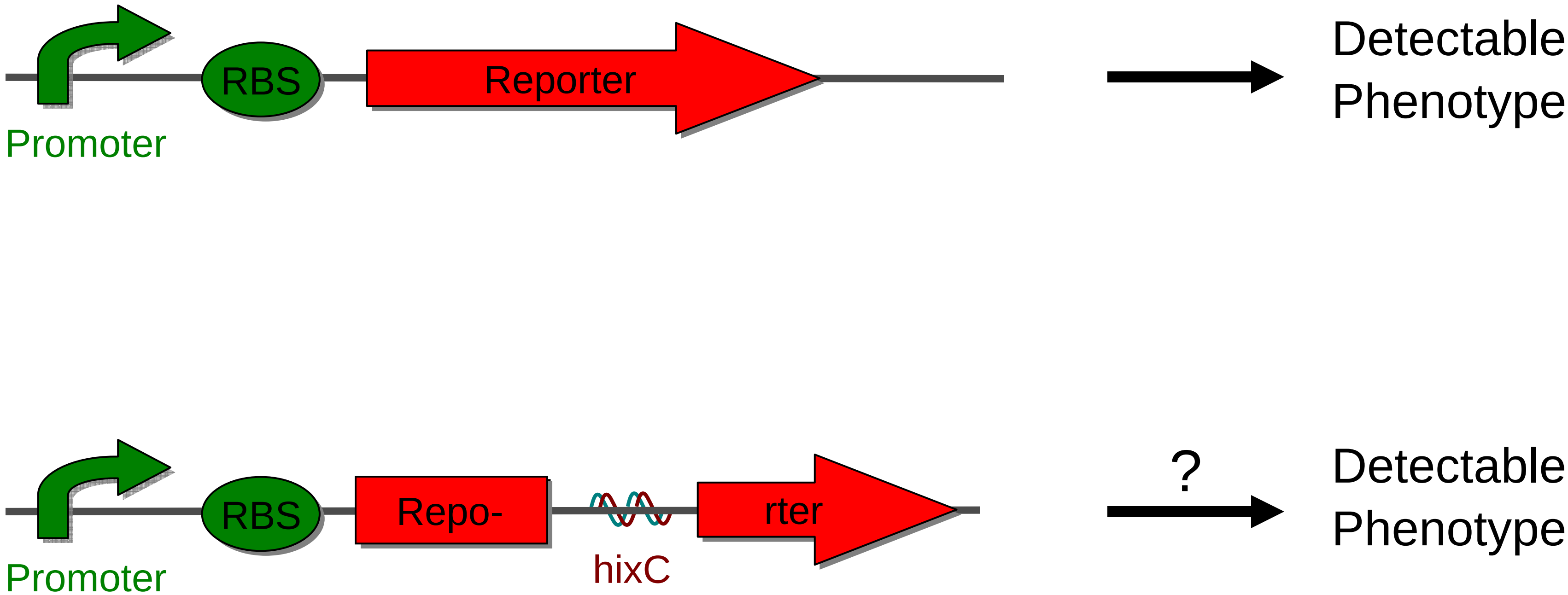


Graph 1

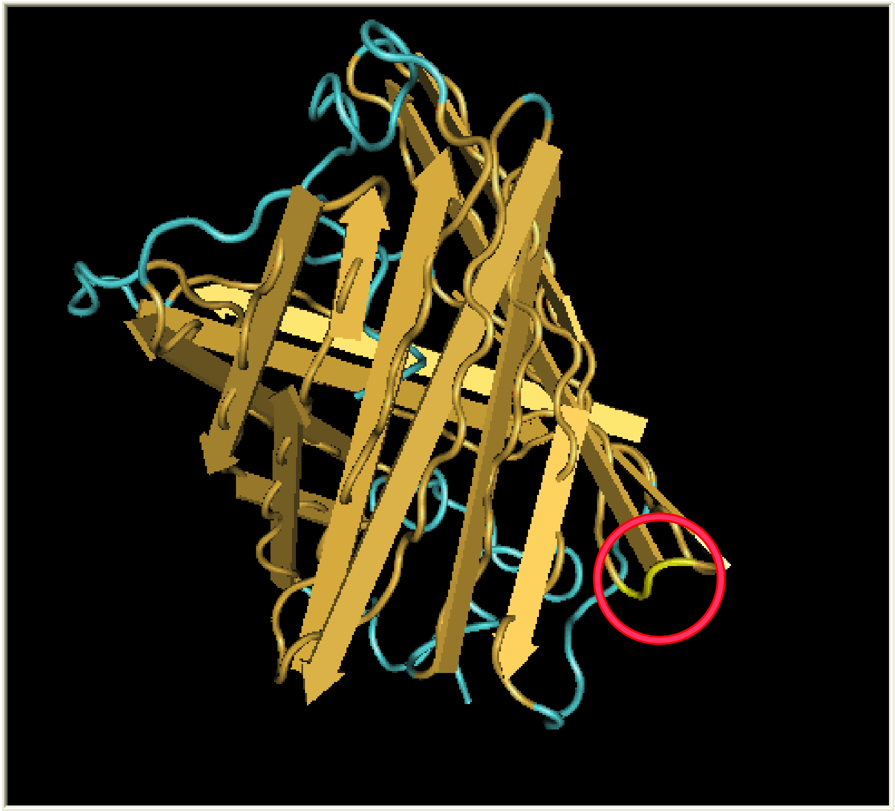


Graph 2

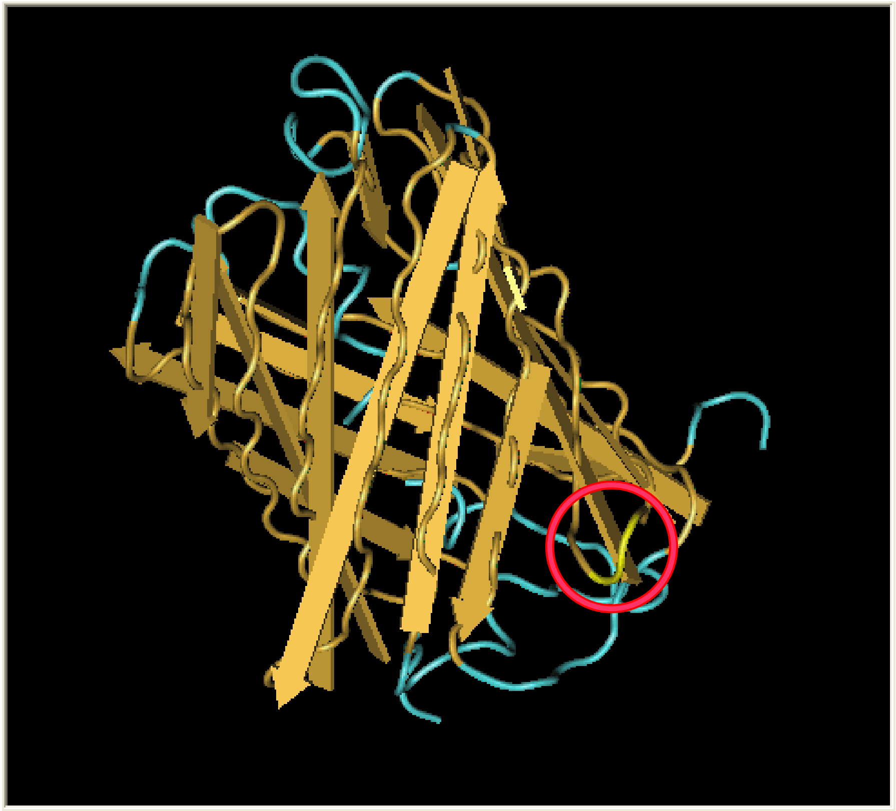
How to Split a Gene



Splitting Reporter Genes

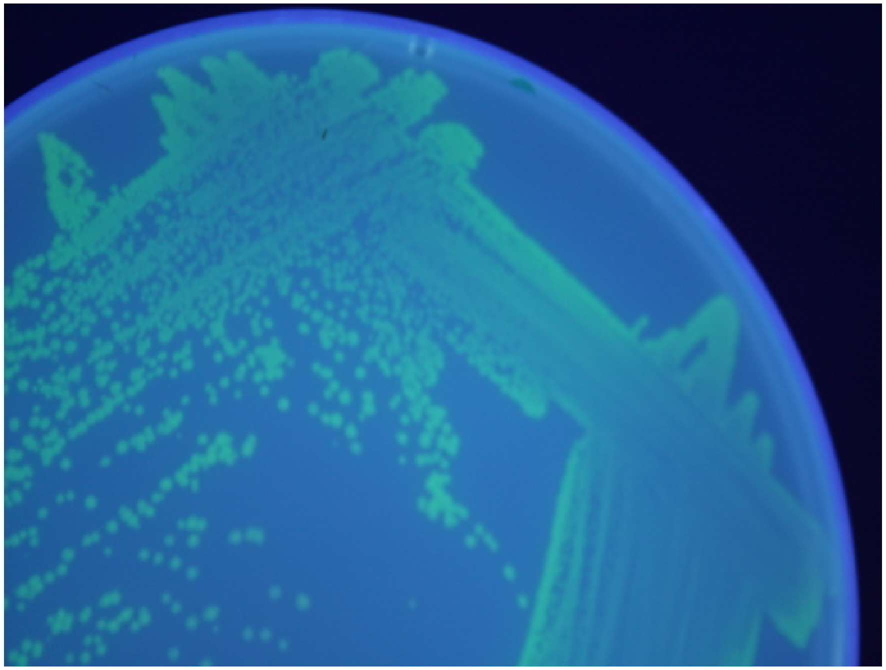


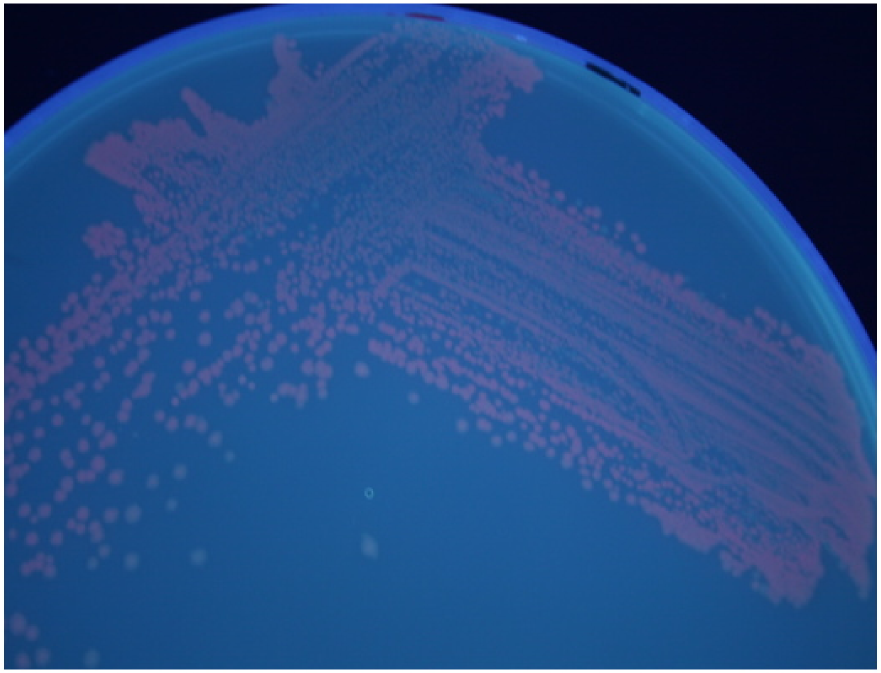
Green Fluorescent Protein



Red Fluorescent Protein

Splitting Reporter Genes





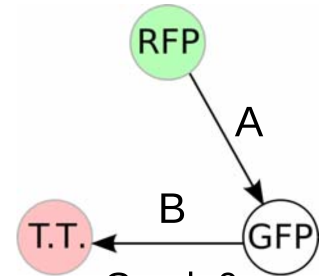
GFP Split by *hixC*

RFP Split by *hixC*

HPP Constructs

Graph 0 Construct:

AB

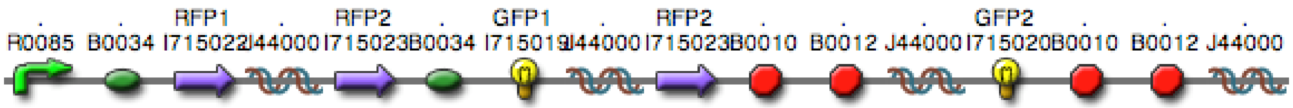


Graph 0

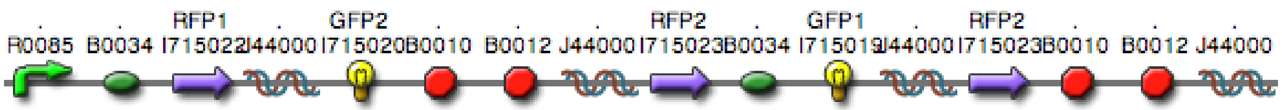
Graph 1 Constructs:

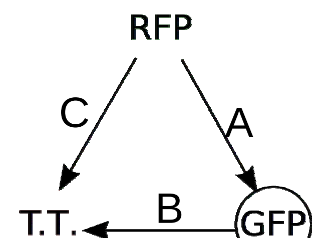
ABC

ACB



BAC

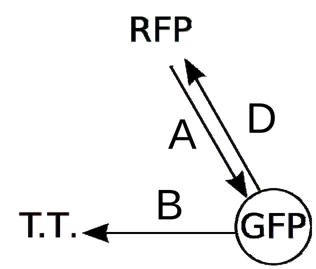




Graph 1

Graph 2 Construct:

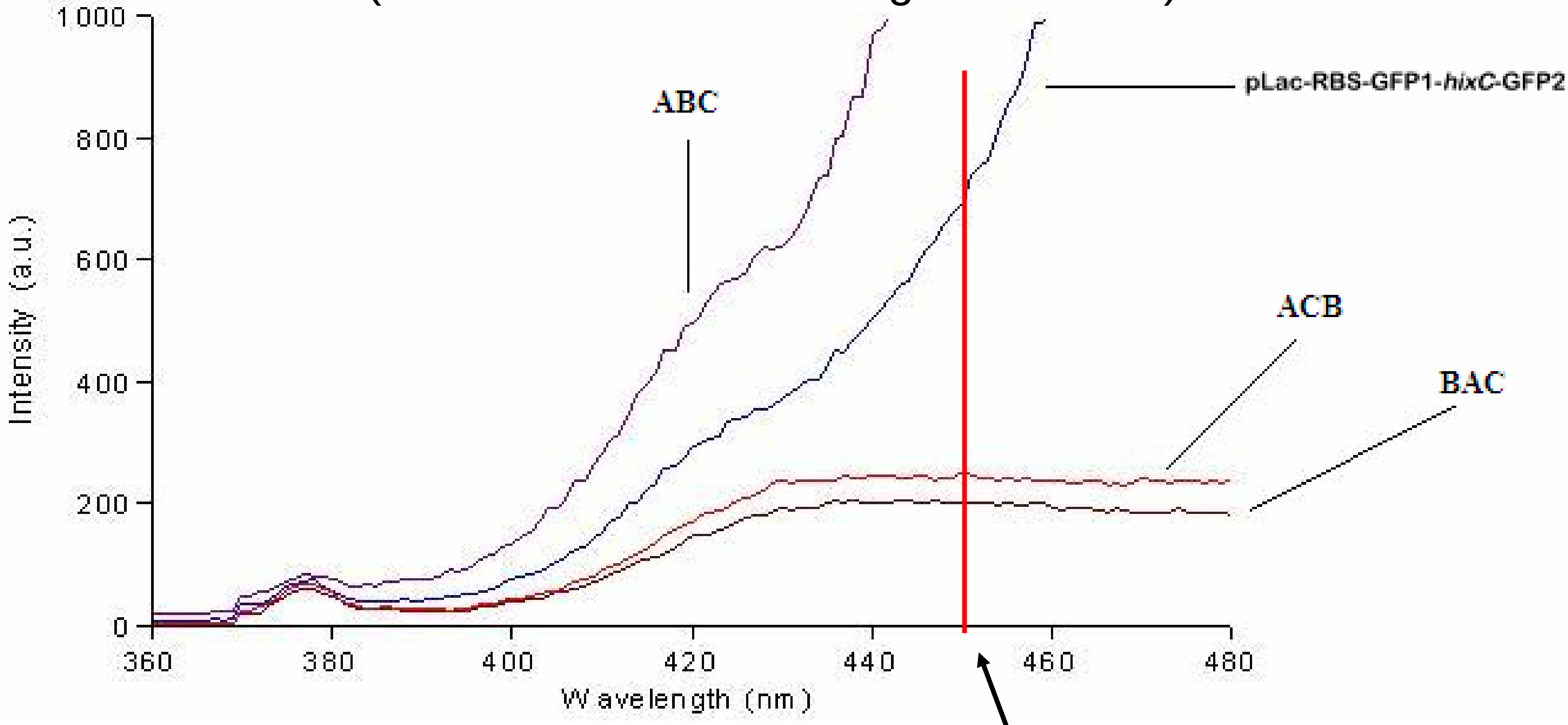
DBA



Graph 2

Measuring Fluorescence

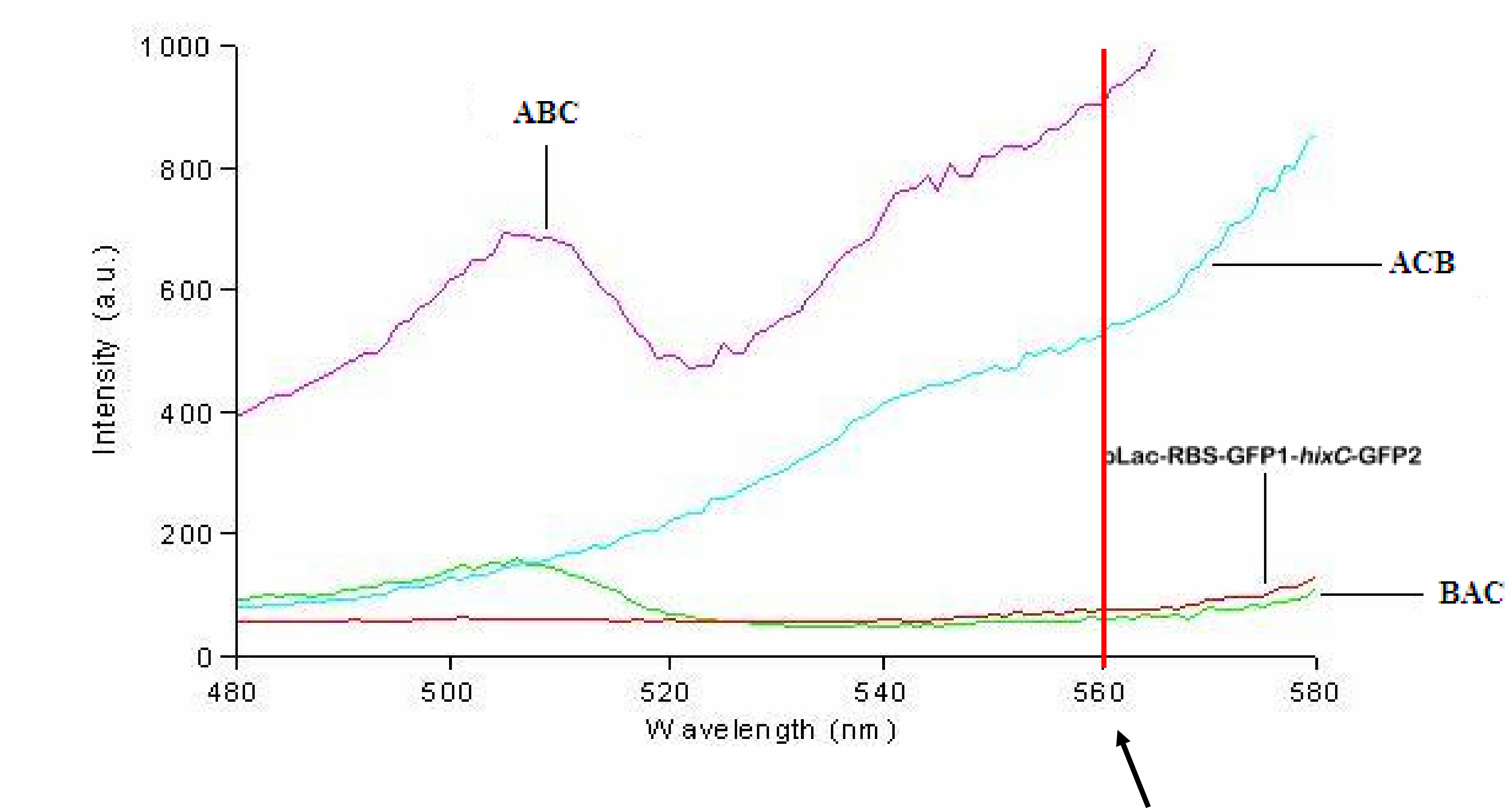
GFP Excitation Spectra for 4 HPP Constructs
(at an Emission Wavelength of 515nm)



450 nm chosen as excitation wavelength to measure GFP

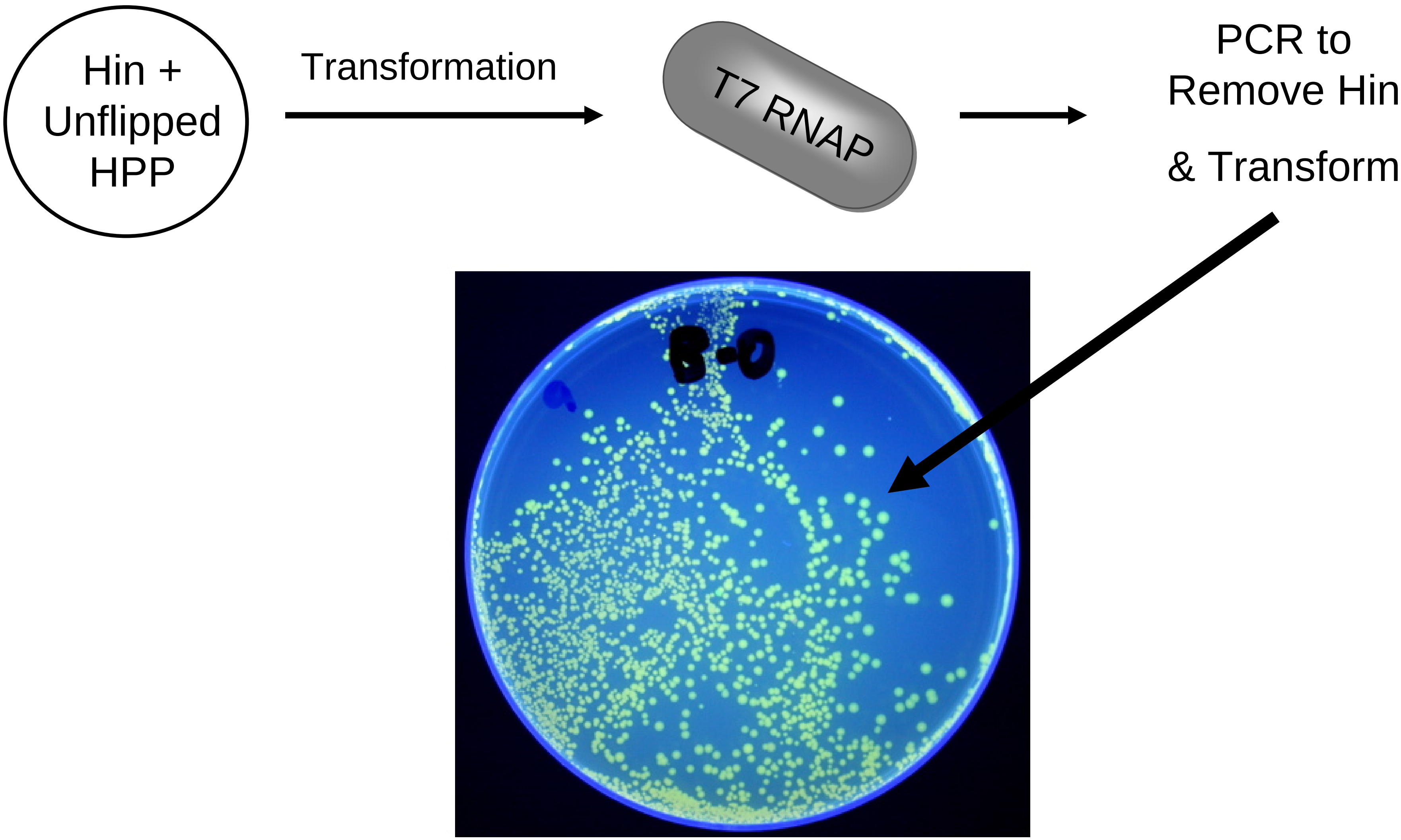
Measuring Fluorescence

RFP Excitation Spectra for 4 HPP Constructs
(at an Emission Wavelength of 608nm)



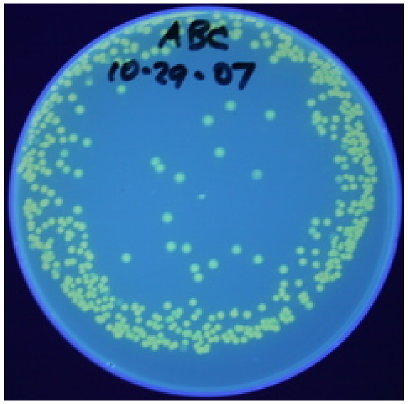
560 nm chosen as excitation wavelength to measure RFP

Coupled Hin & HPP Graph

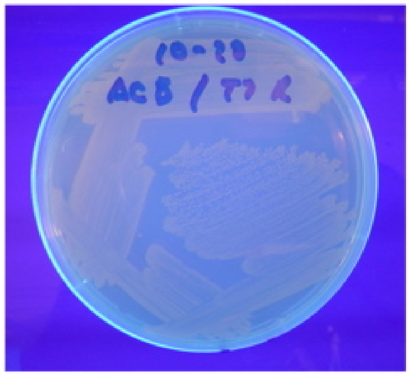


Flipping Detected by Phenotype

ABC
(Yellow)



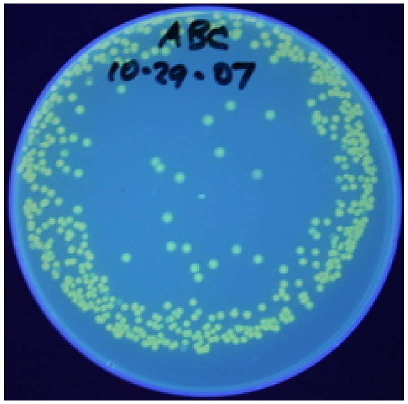
ACB
(Red)

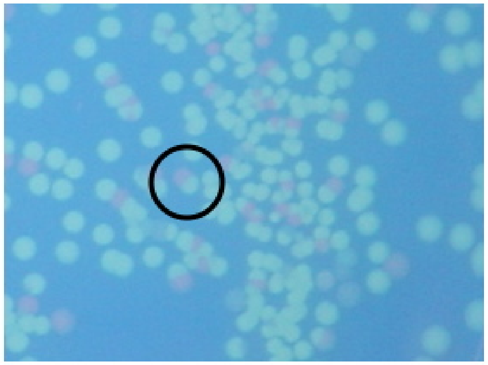


BAC
(None)

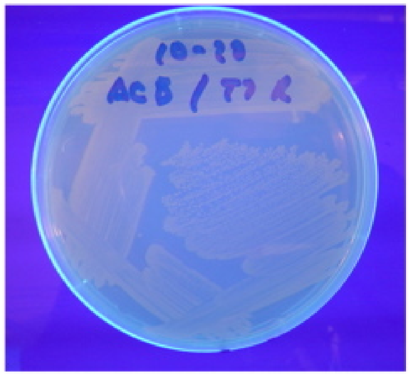
Flipping Detected by Phenotype

ABC
(Yellow)

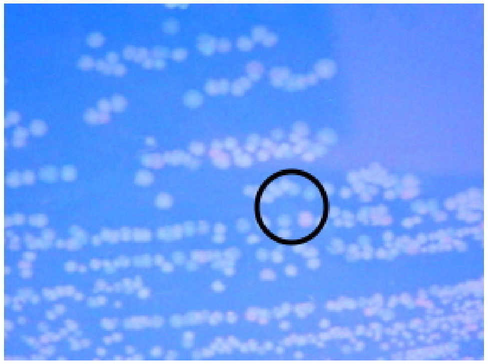




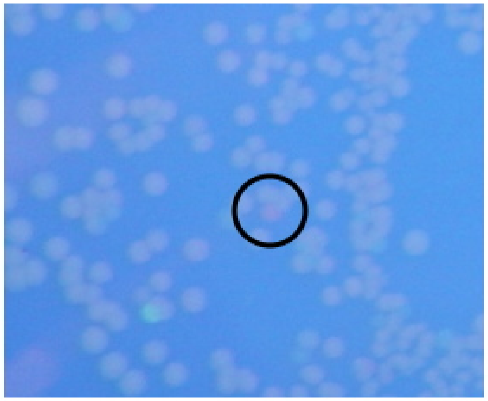
ACB
(Red)



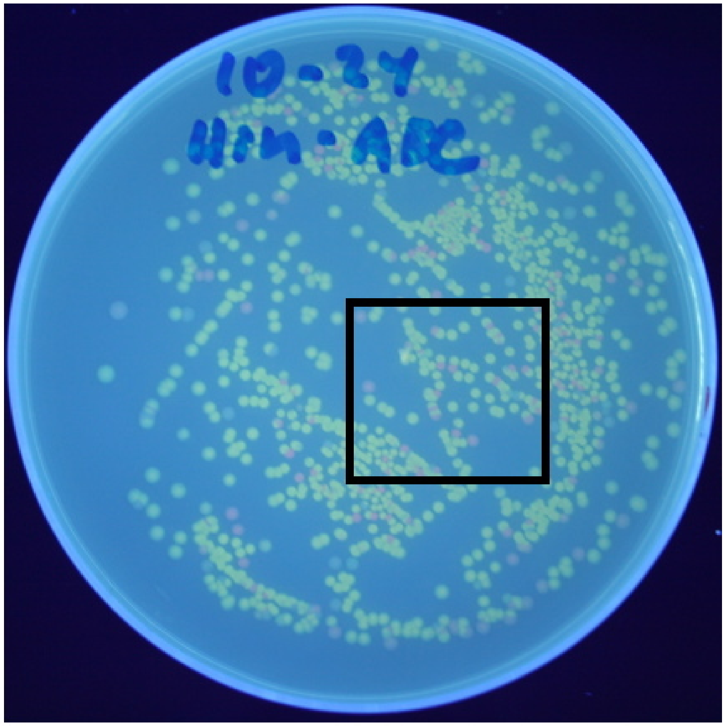
Hin-Mediated
Flipping

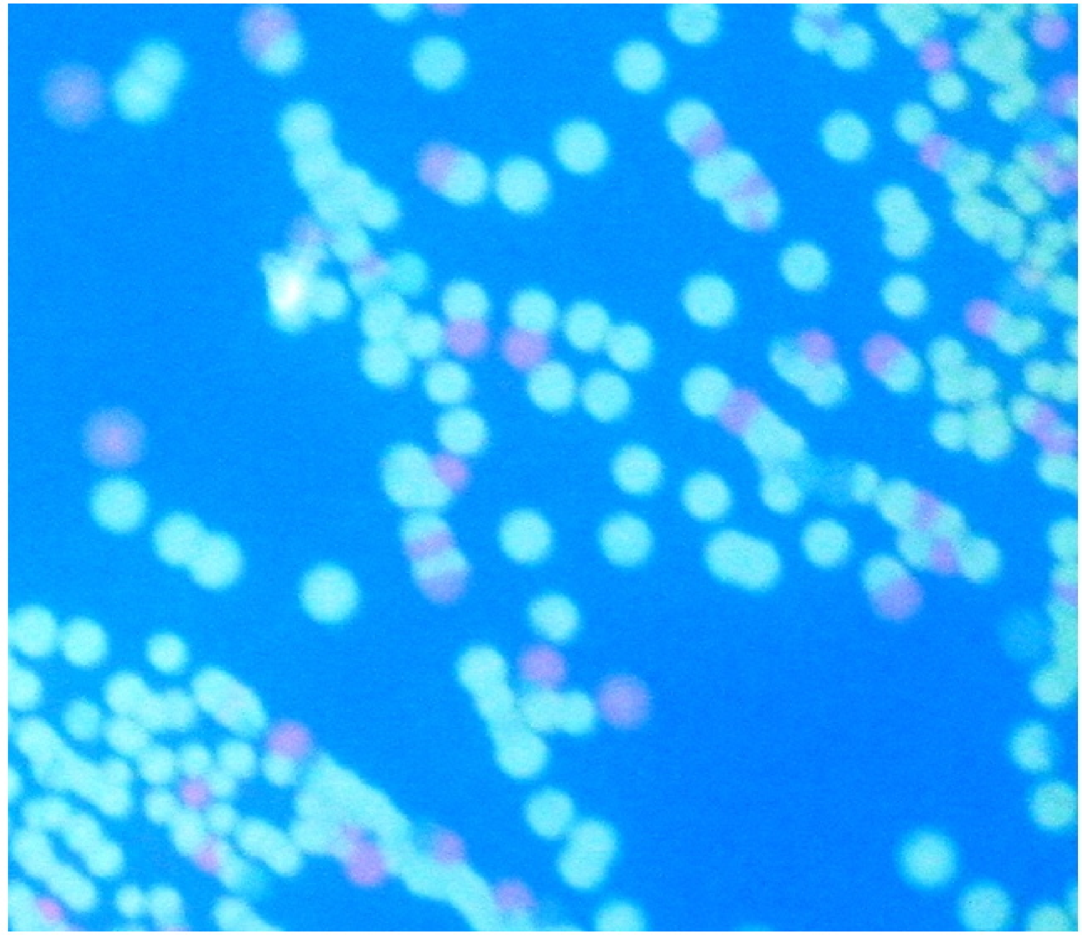


BAC
(None)



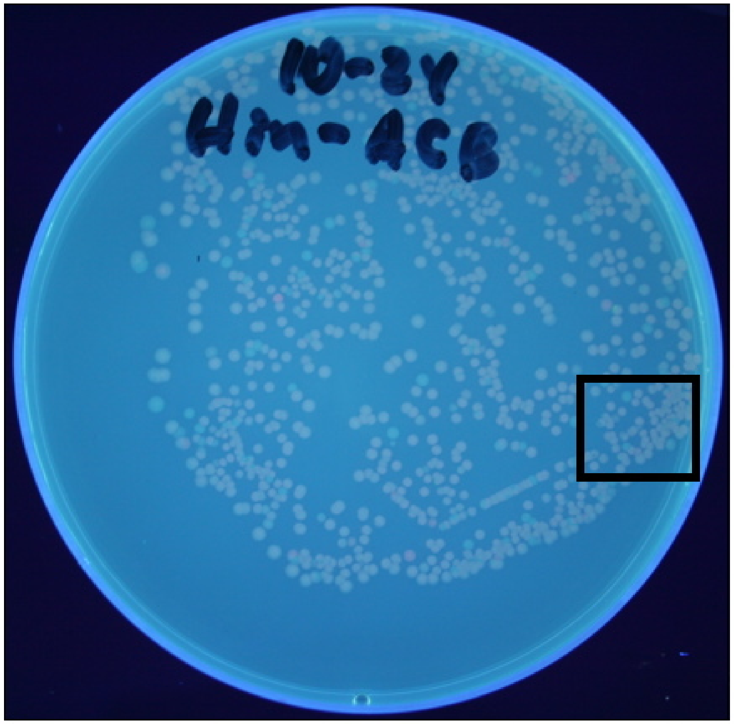
ABC Flipping





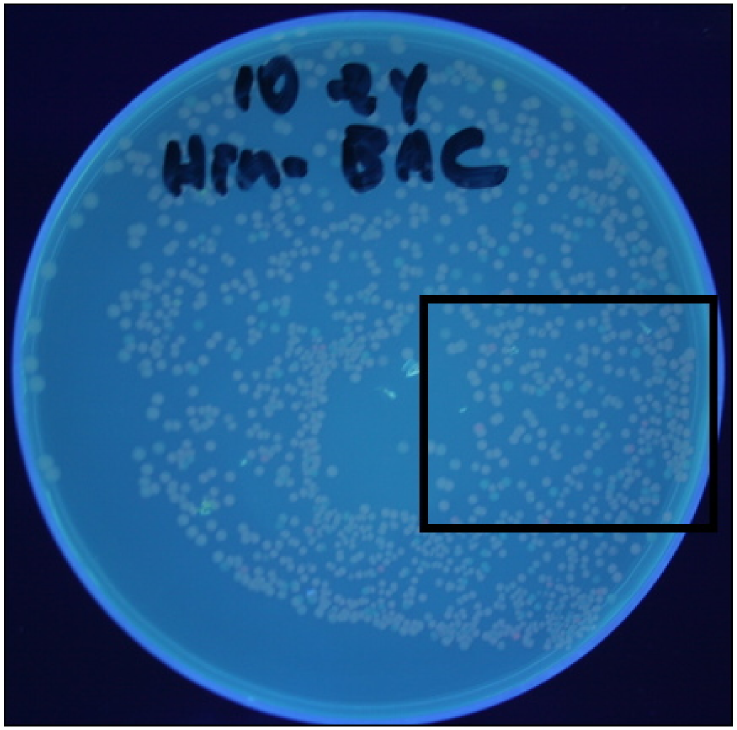
Yellow —Hin→ Yellow, Green, Red, None

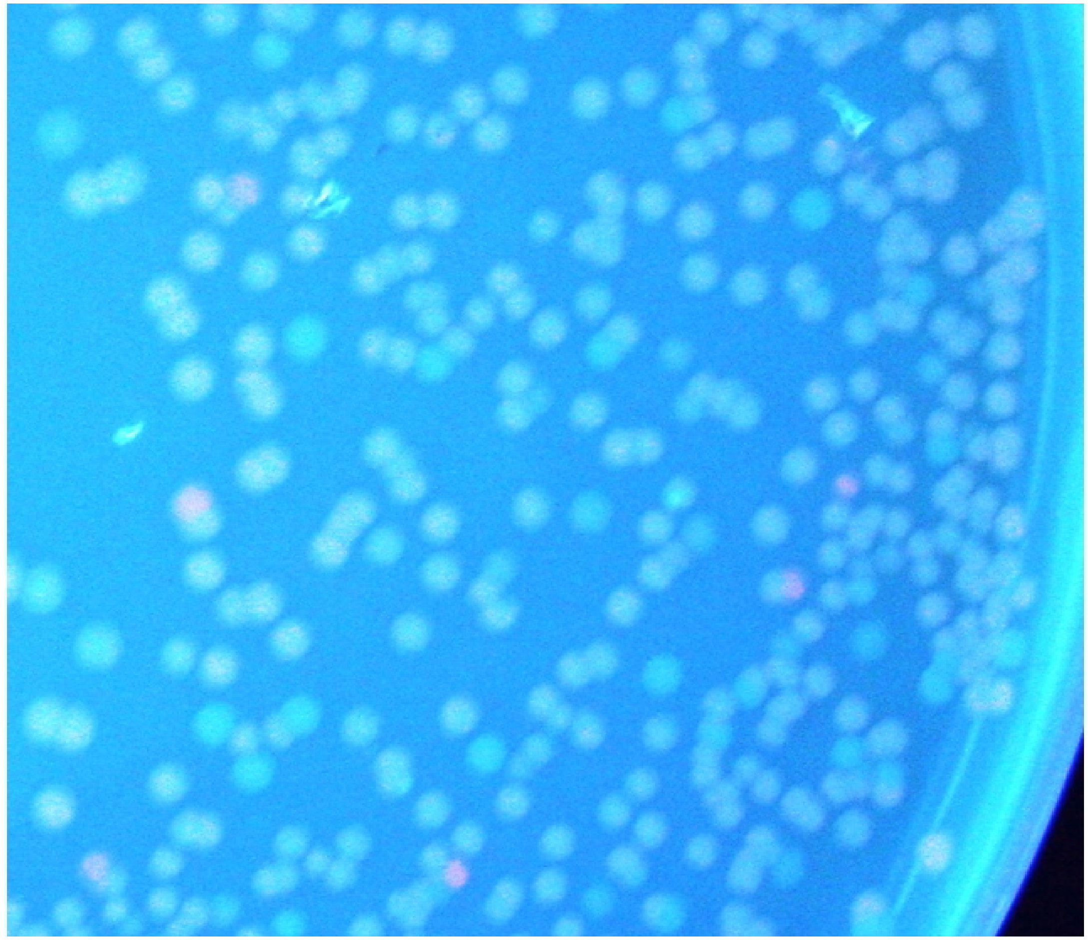
ACB Flipping



Red —Hin→ Yellow, Green, Red, None

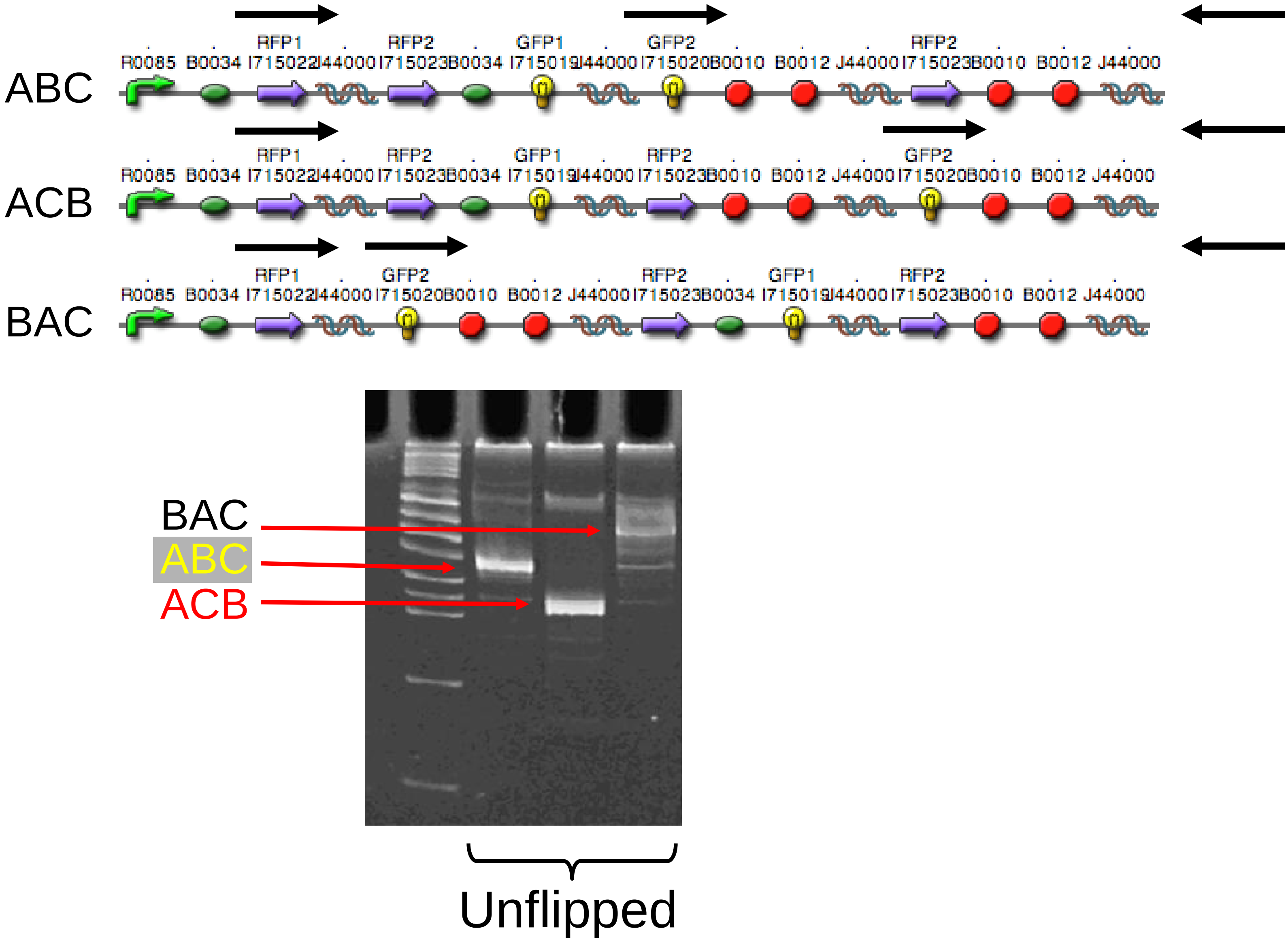
BAC Flipping



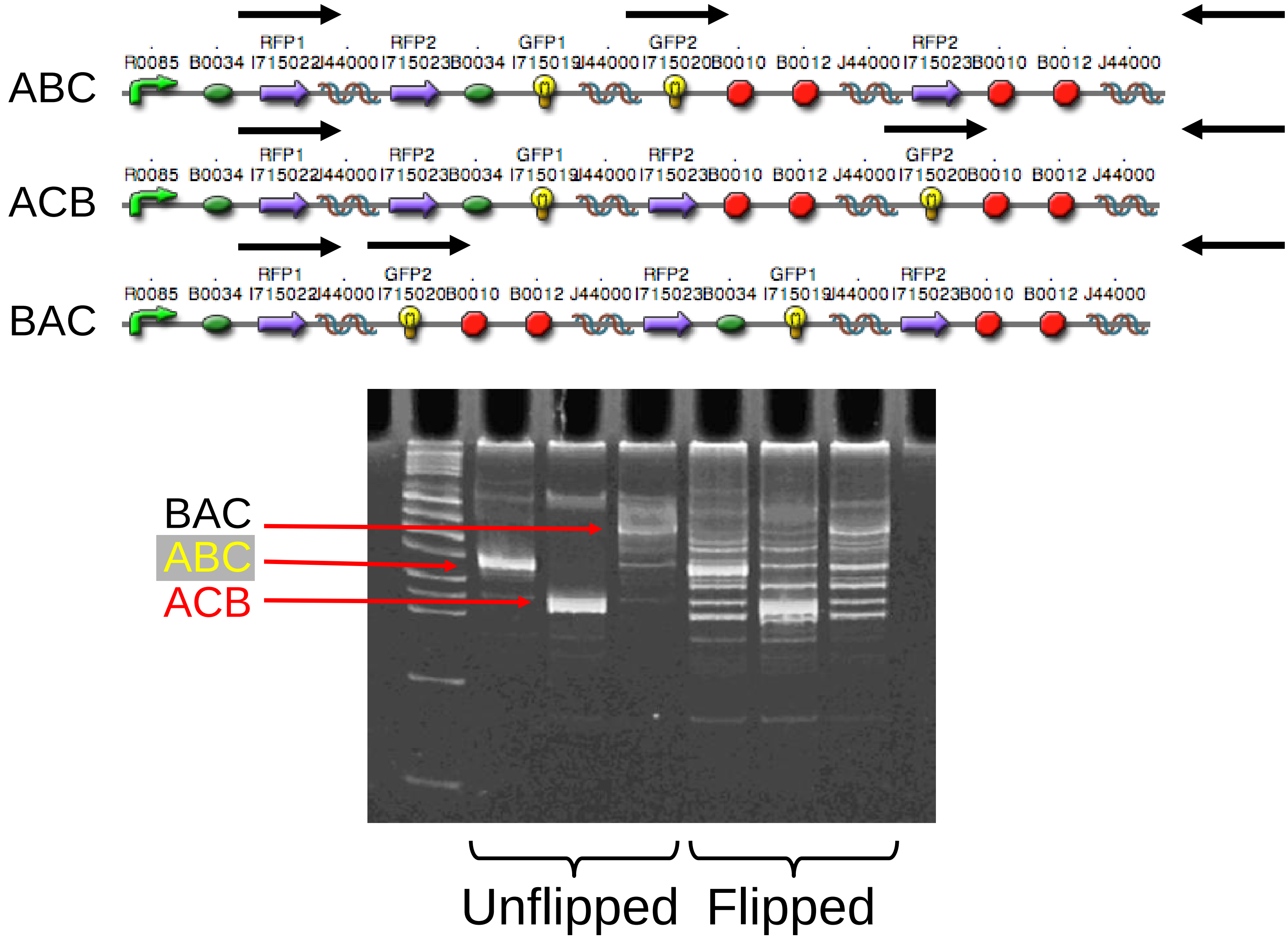


None —Hin→ Yellow, Green, Red, None

Flipping Detected by PCR

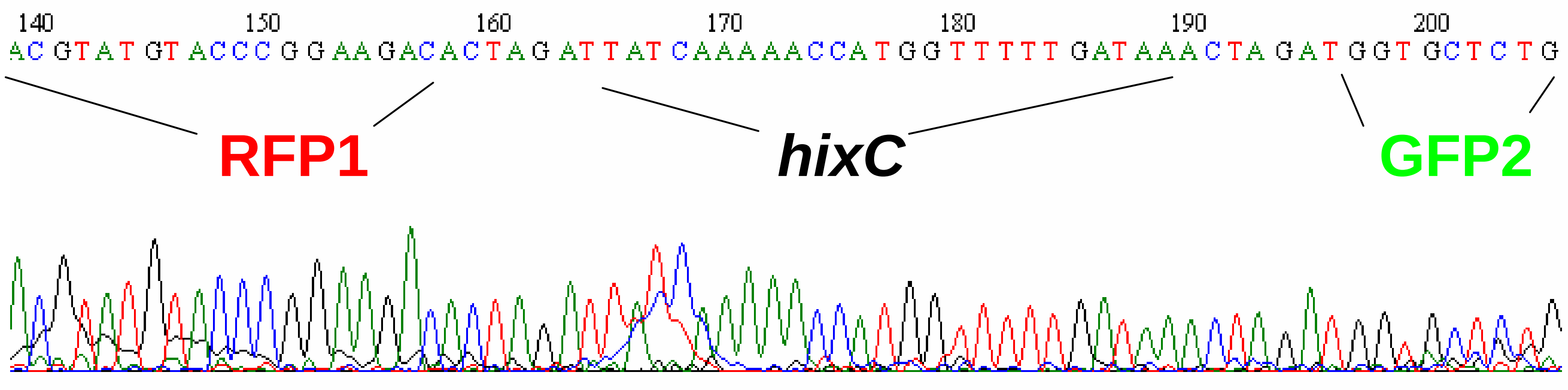


Flipping Detected by PCR



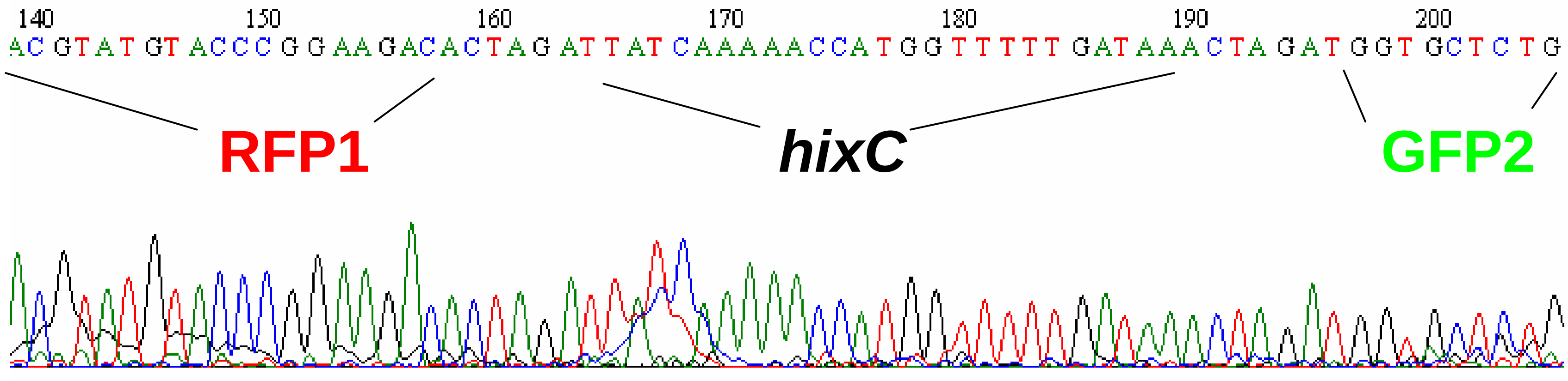
Flipping Detected by Sequencing

BAC



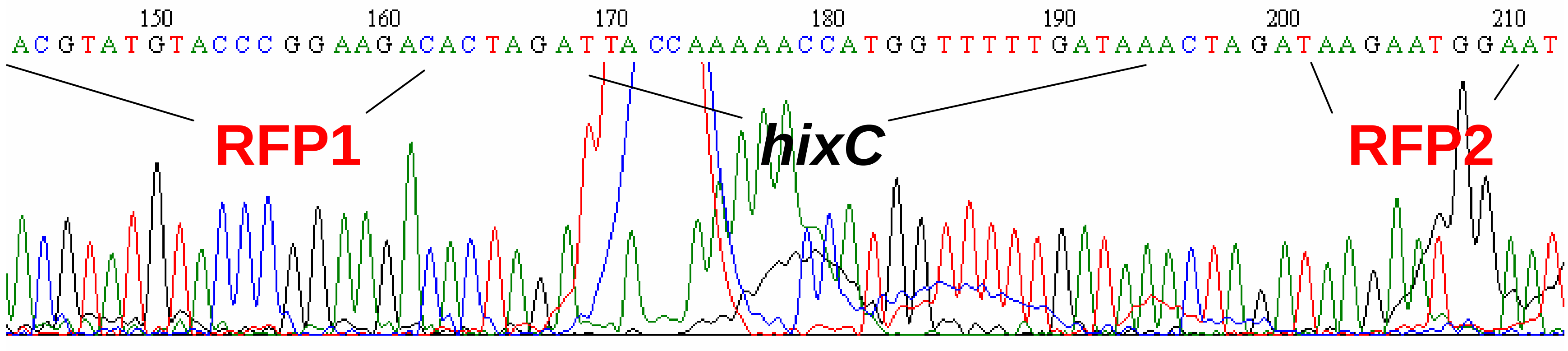
Flipping Detected by Sequencing

BAC



Hin

Flipped-BAC



Conclusions

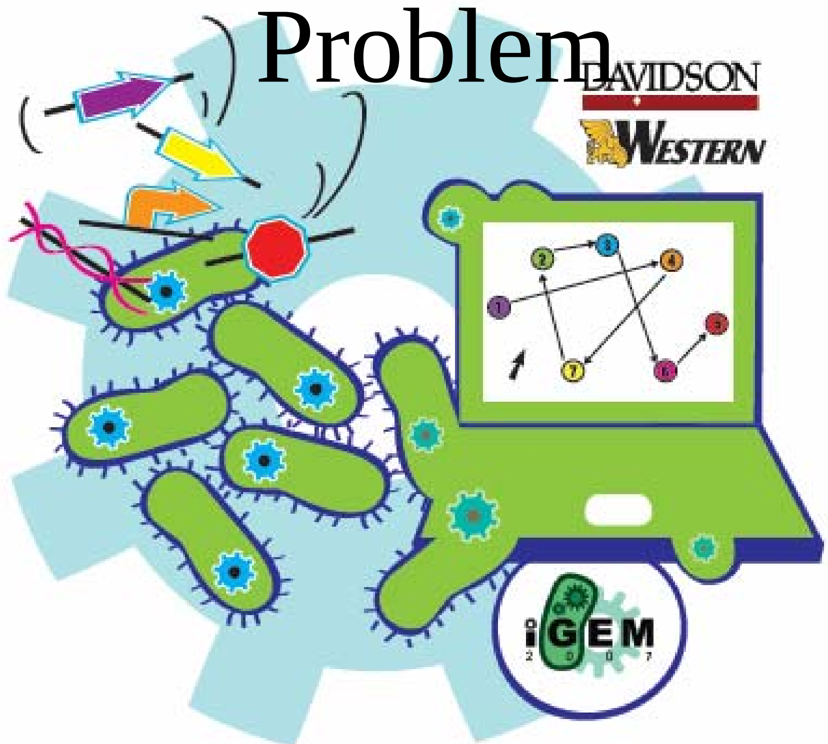
- Modeling revealed feasibility of our approach
- GFP and RFP successfully split using *hixC*
- Added 69 parts to the Registry
- HPP problems given to bacteria
- Flipping shown by fluorescence, PCR, and sequence
- Bacterial computers are working on the HPP and may have solved it

Living Hardware to Solve the Hamiltonian Path Problem

Students: Oyinade Adefuye, Will DeLoache, Jim Dickson, Andrew Martens, Amber Shoecraft, and Mike Waters, Jordan Baumgardner, Tom Crowley, Lane Heard, Nick Morton, Michelle Ritter, Jessica Treece, Matt Unzicker, Amanda Valencia

Faculty: Malcolm Campbell, Todd Eckdahl, Karmella Haynes, Laurie Heyer, Jeff Poet

Living Hardware to Solve the Hamiltonian Path Problem



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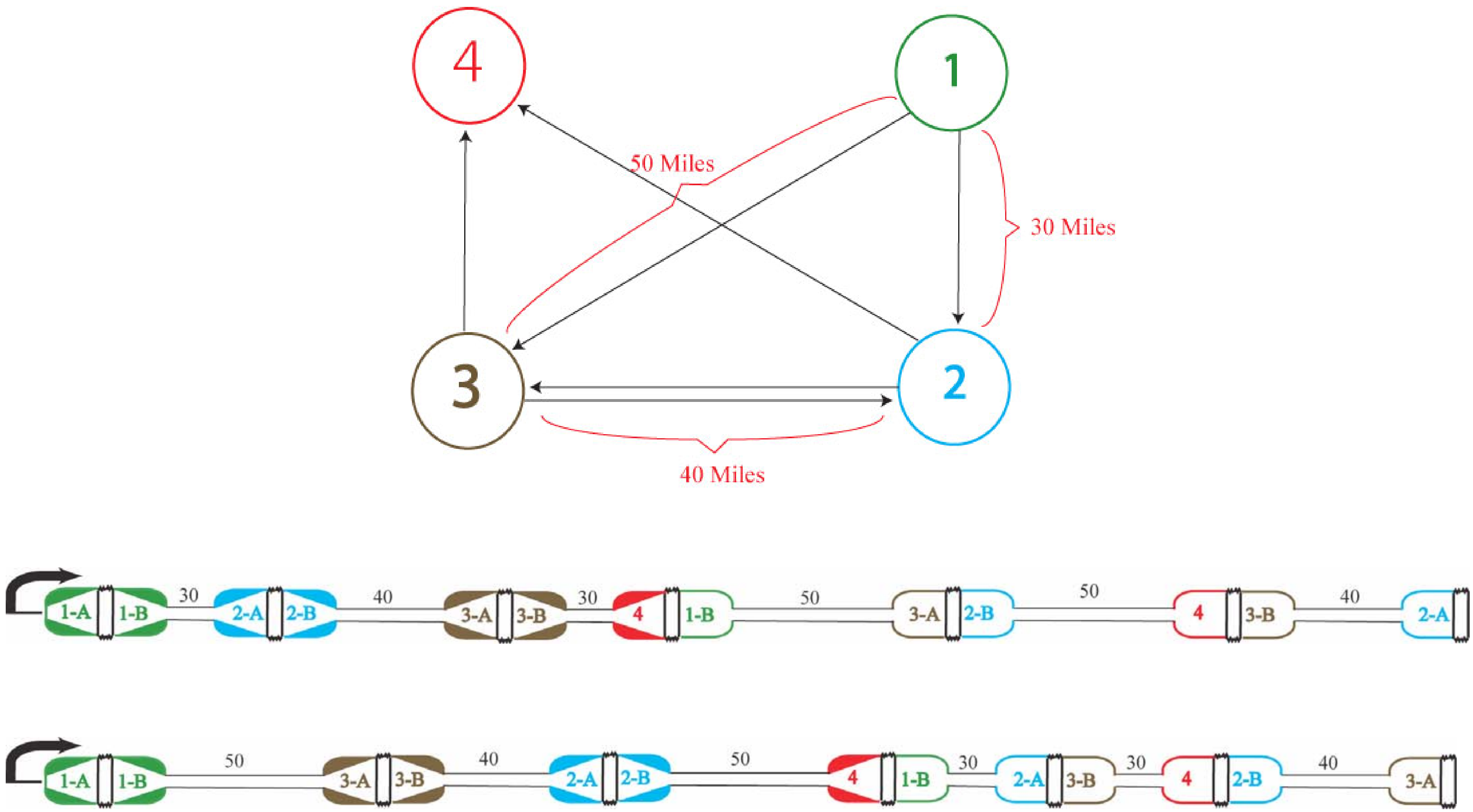
Extra Slides

Traveling Salesperson Problem



Another Gene-Splitting Method

RBS + ATG ------*hixC*----- 1 bp + Reporter Gene1

Front Half — Back Half

RBS + ATG + 1bp ------*hixC*----- Reporter Gene2

Front Half — Back Half

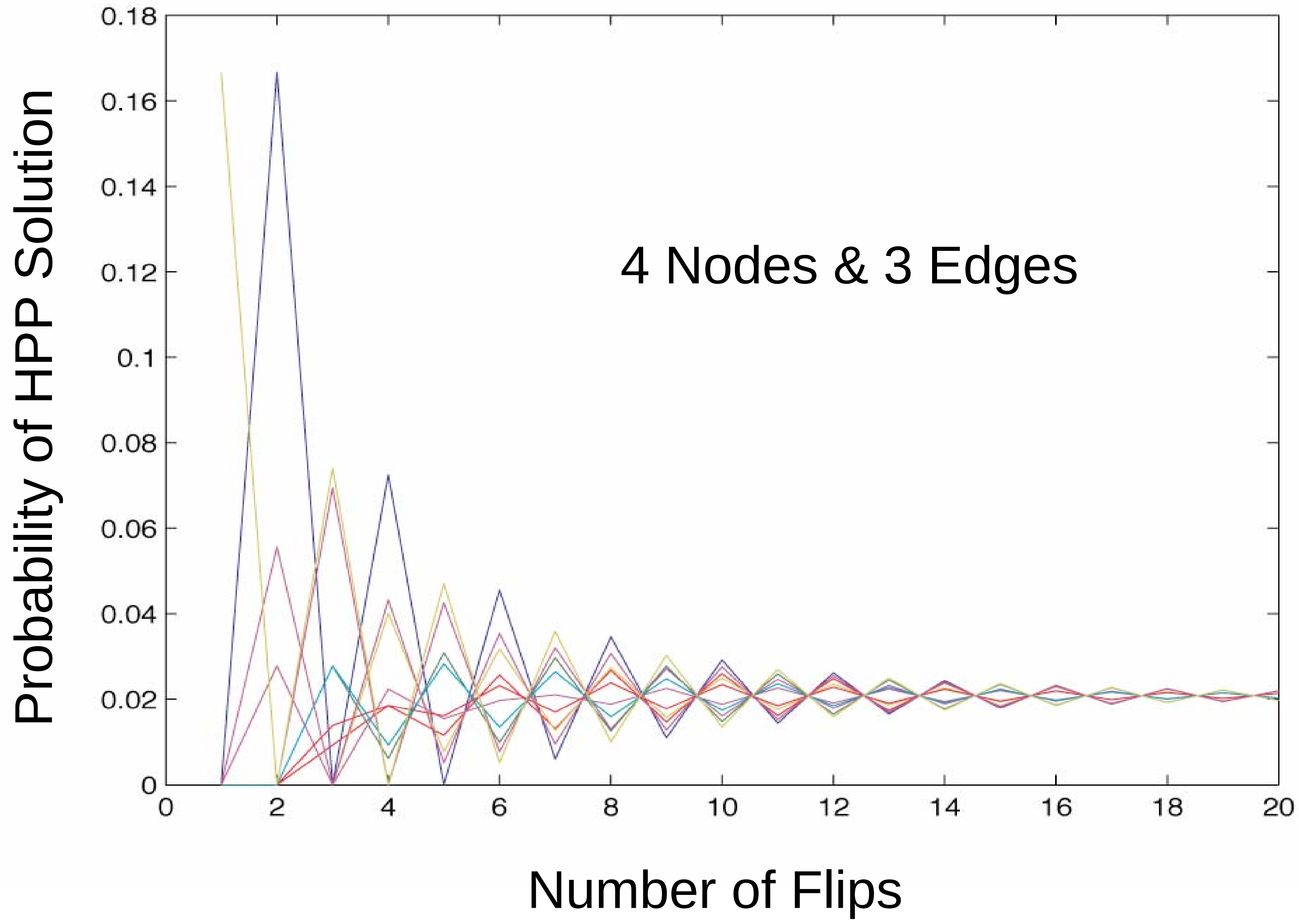
Other Attempts at Gene Splitting

Reporter Gene	Status
Kanamycin Resistance	Failed
Tetracycline Resistance	Failed
Chloramphenicol Resistance	Undetermined (Issues in building)
Cre Recombinase	Undetermined (Issues in testing)

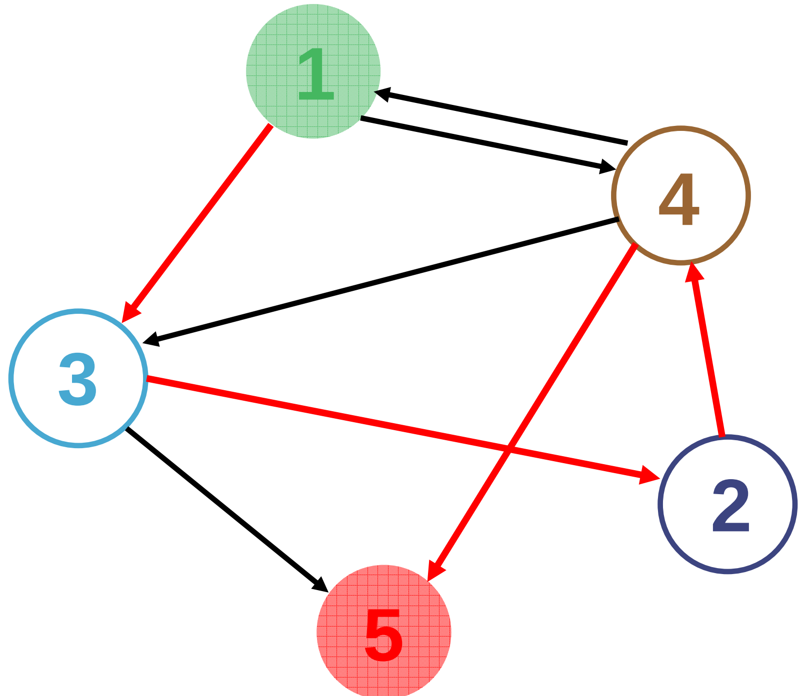
Normalized Fluorescence Measurements

Construct	Observed Color on UV Box	Green (450/515)	Red (560/608)
pLac-RBS-RFP	Red	7	263
pLac-RBS-RFP-RBS-GFP	Red	144	370
pLac-GFP1-*hixC*-GFP2	Green	136	0
pLac-RBS-RFP1-*hixC*-RFP2	None	0	147
pLac-RBS-GFP1-*hixC*-RFP2	None	11	2
pLac-RBS-RFP1-*hixC*-GFP2	None	13	2
AB (R1-*hixC*-R2-G1-*hixC*-G2)	Green	72	18
ABC (R1-*hixC*-R2-G1-*hixC*-G2)	Yellow	340	255
ACB (R1-*hixC*-R2-G1-*hixC*-R2)	Red	1	143
BAC (R1-*hixC*-G2)	None	11	3
DBA (R1-hixC-G2)	Hybrid green	15	3

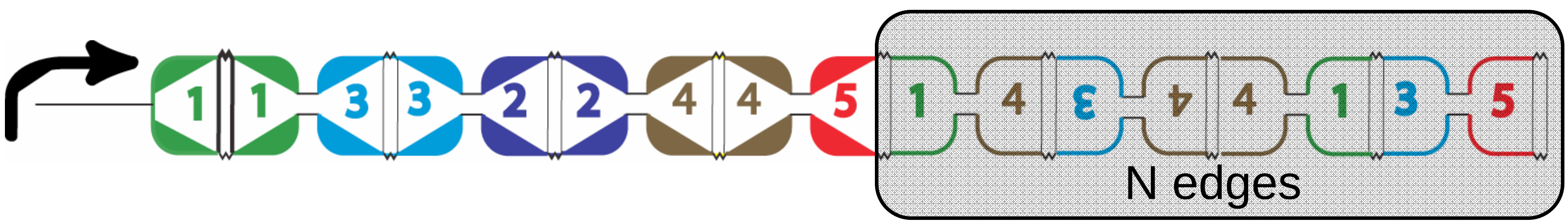
Starting Arrangement



True Positives



Elements in the shaded region can be arranged in any order.



Number of True Positives = $N! * 2^N$