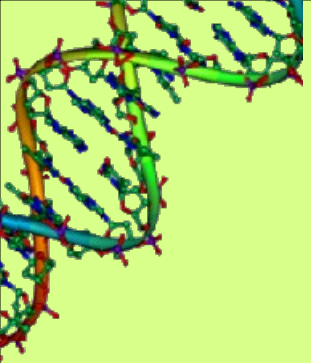
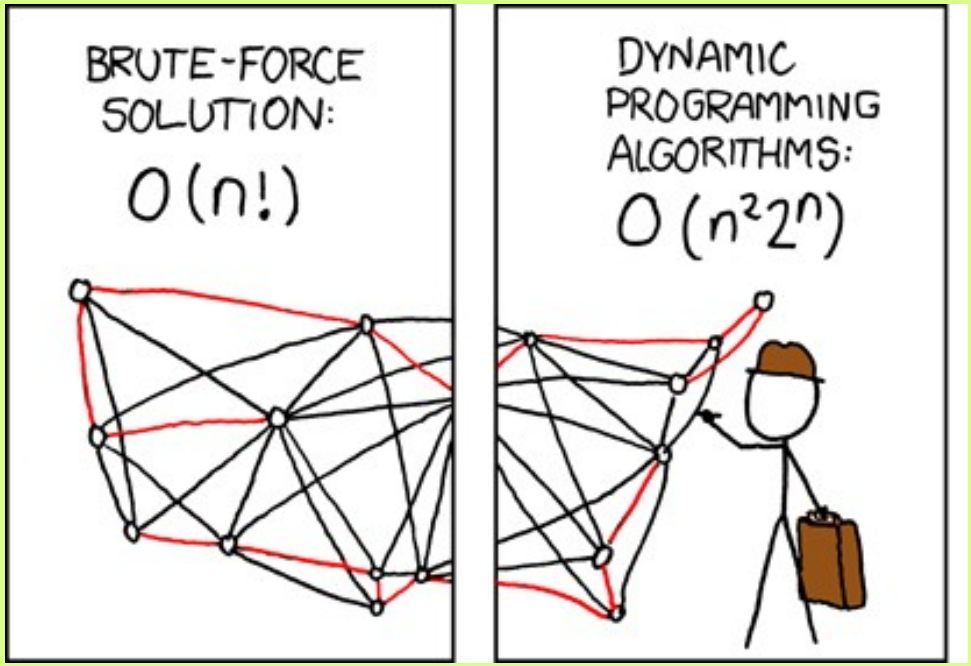


# Sweetening Probiotic Curd, Controlled by a pseudo-AND gate

*Presented by IIT_Madras at the iGEM 2010 Jamboree*




The travelling salesman

# Waiting for a solution

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NEW DELHI, January 19, 2010

## Bt Brinjal safe for humans, says science ministry

P. SUNDERARAJAN

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Environmen: MinisterJairam Ramesh interacts with protesters as he arrives to attend a consultation on Bt brinjal in Ahmedabad on Tuesday. The government is organizing a series of public consultations to decide on the approval of India's first GM crop. AP

RELATED

Even as the debate over the release of Bt Brinjal for commercial cultivation continues unabated, Union Science and Technology Minister, Prithviraj Chavan on Tuesday

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NEW DELHI, January 30, 2010

## Opposing Bt brinjal, fifty go on fast

STAFF REPORTER

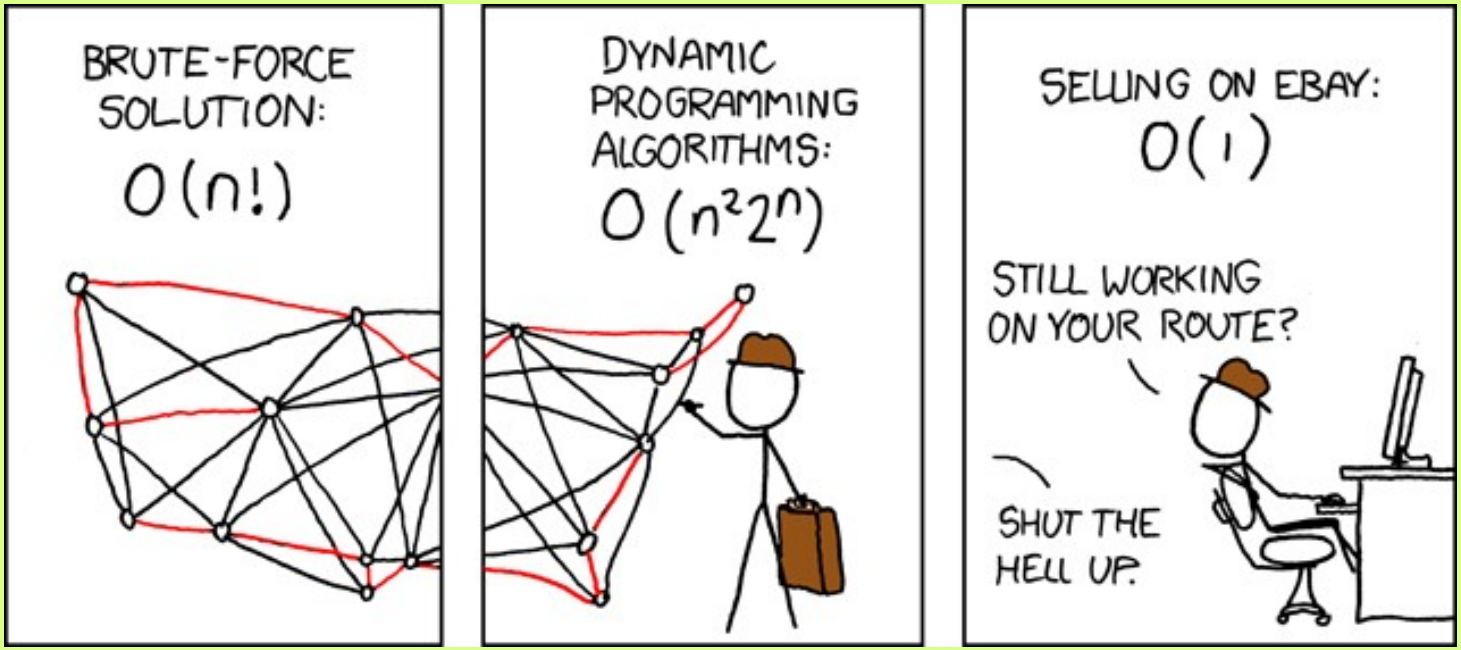
SHARE · PRINT · T+


Citizens taking out a candle light vigil to observe National Day of Fast on the occassion of Gandhi's death anniversary to oppose the introduction of genetically modified BT Brinjal, at Jantar Mantar in New Delhi on Saturday. Photo: S. Subramanium The Hindu

RELATED

NEWS

To protest against the introduction of Bt brinjal in Indian markets, over 50 people went on fast at Jantar Mantar here on Saturday.



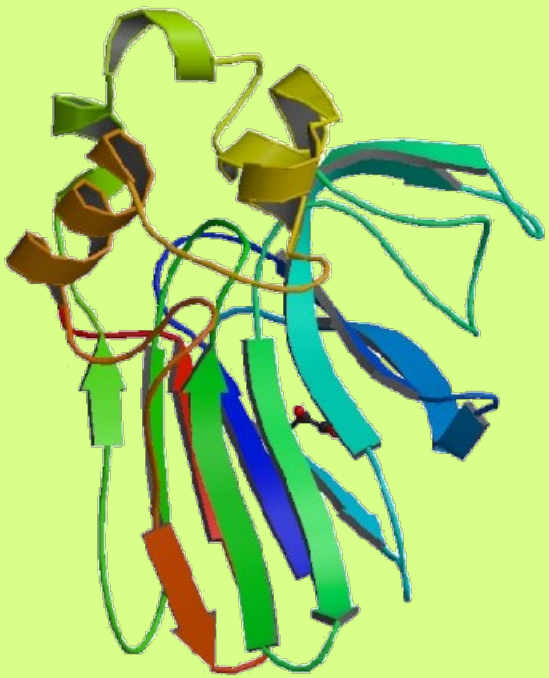

The travelling salesman

# Sweet genes?

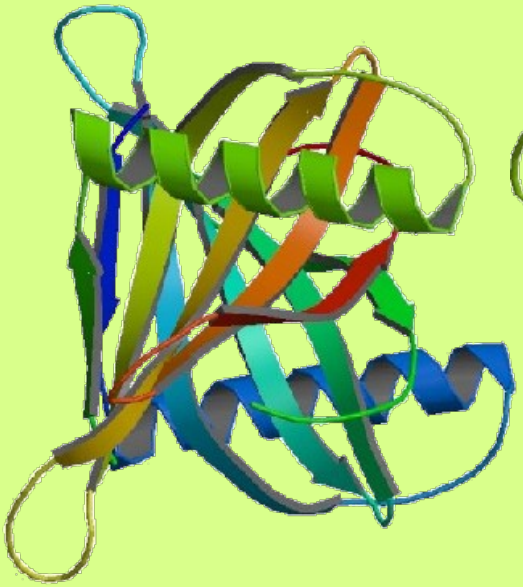
- Many times the 'sweetness' of sugar
- Delayed onset of sweetness and lingering aftertaste.
- Small peptides optimised for stability and expression in bacterial systems.

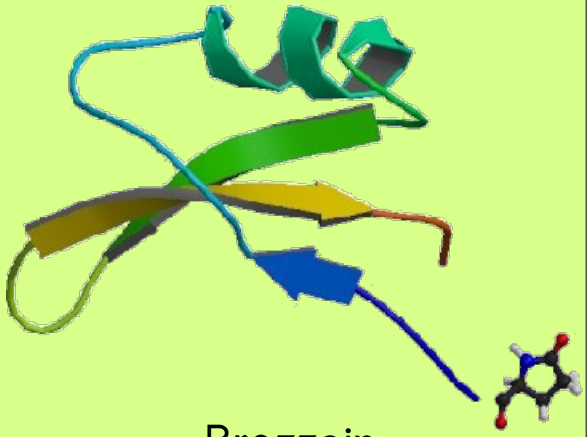
MNEI

Thaumatin



MNEI

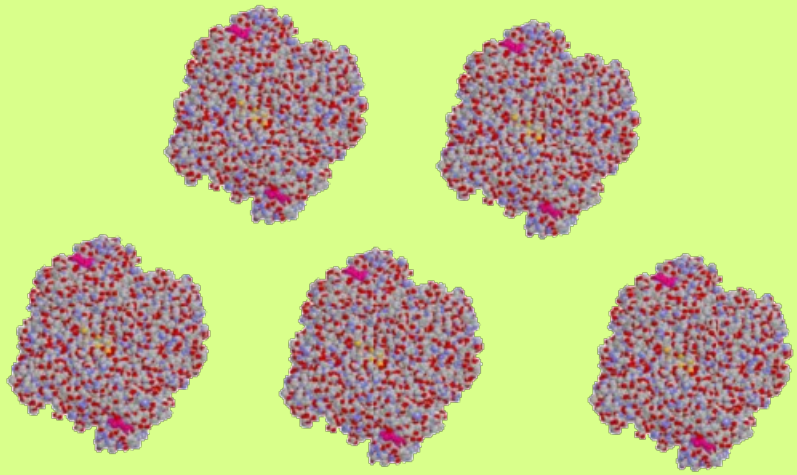




Brazzein

# An Engineer's Biology

- Regulation vs Expression?
- One Input gives ON / OFF control, Multiple inputs?
- Creating the window; the AND-gate.



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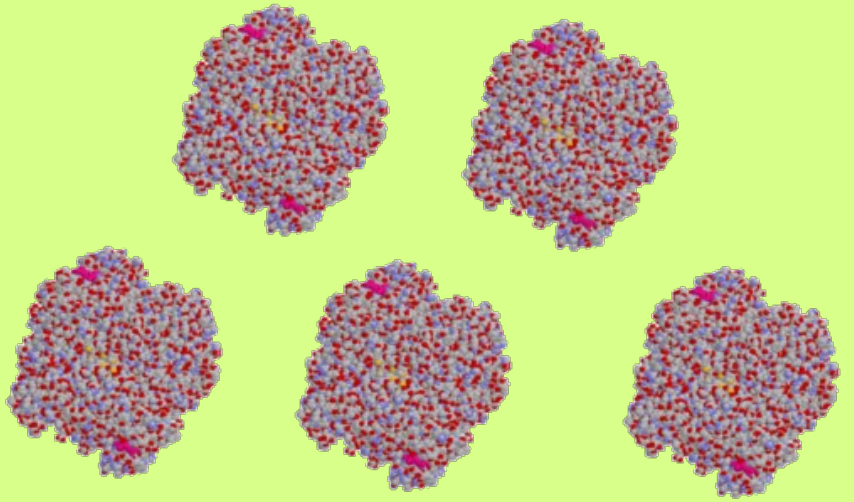

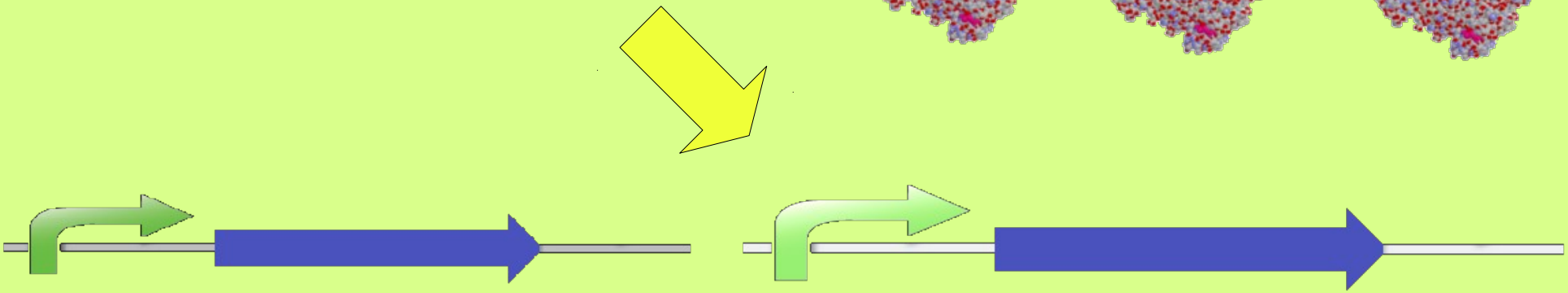

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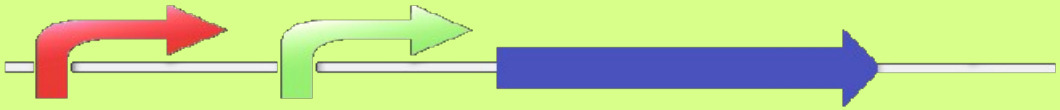
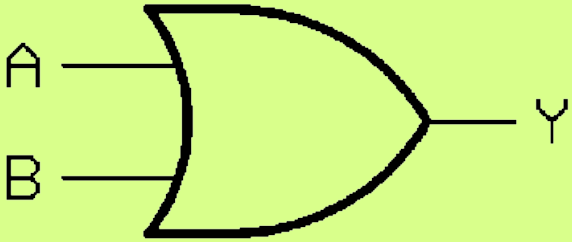
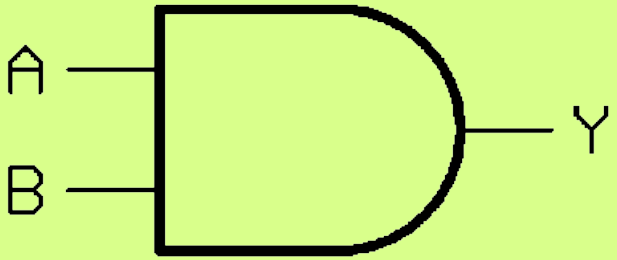
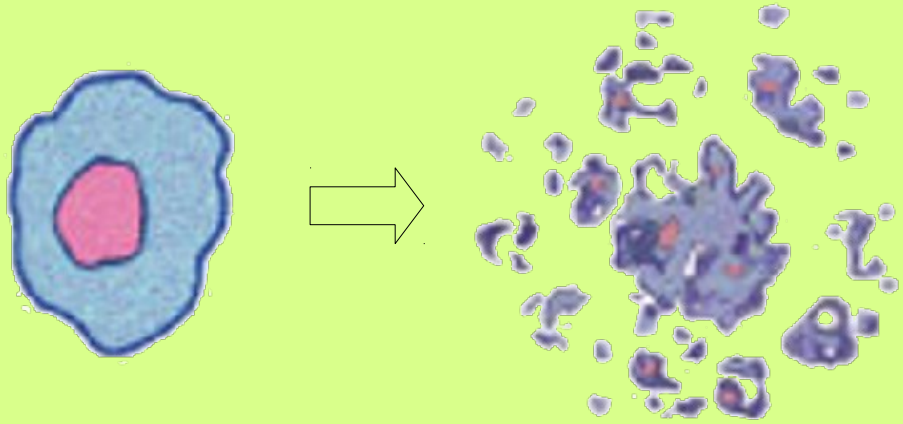




# The 'Undo' button

- The bane of GM
- Death to the bacteria
- The undo?



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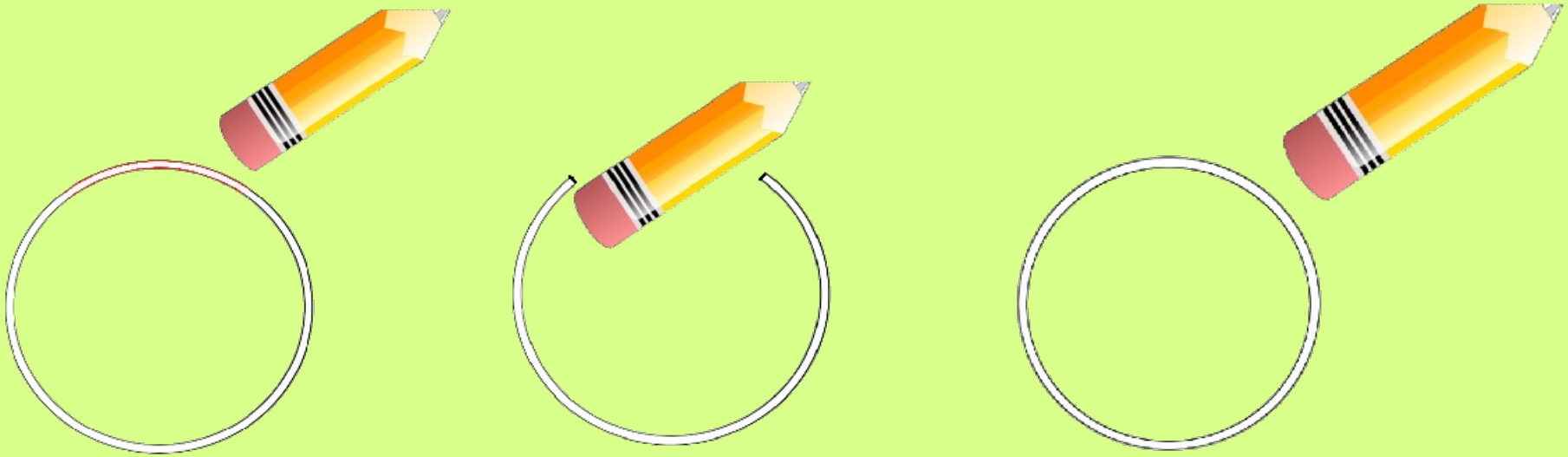
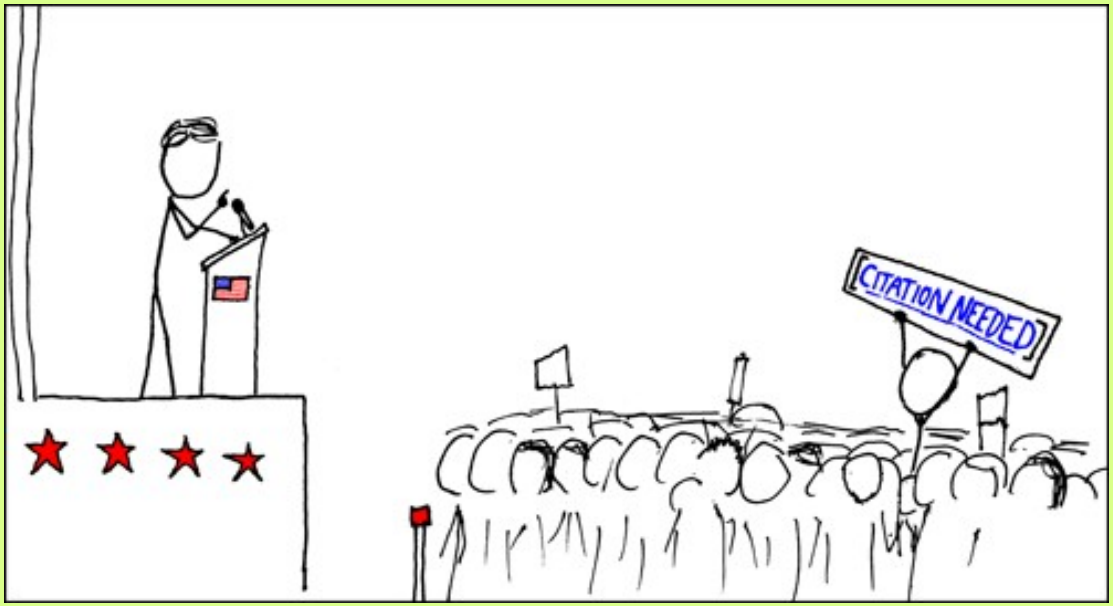



CITATION NEEDED

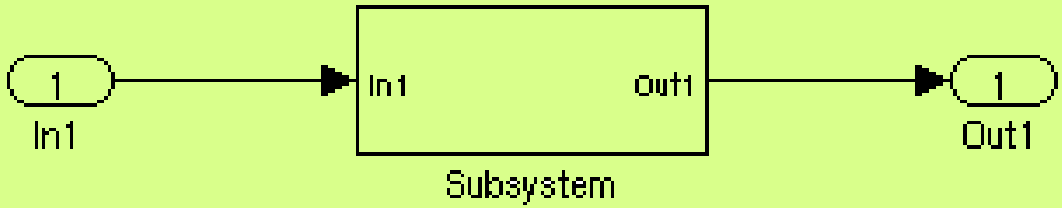
# And the protein is ...

- Choice is not always a good thing.
- Short and sweet
- Monellin
  - 98 amino acids
  - Optimised single chain peptide
  - Temperature and pH stability

| Protein | Relative Sweetness | pH stable | Temp stable |
|---|---|---|---|
| Brazzein | 800x | Yes | Yes |
| Mabinlin 2 | 10x | No | Yes |
| Thaumatin | 2000x | Yes | Yes |
| Monellin (MNEI) | 2000x | Yes | Yes |

# Controlling the system

- And the Inputs are ...
- Smart systems cue themselves; human-proofing



# Controlling the system

| -?- | | Name | Description | Promoter Sequence | Positive Regulators | Negative Regulators |
|---|---|---|---|---|---|---|
| A | | BBa_I1051 | Lux cassette right promoter | ...gttatagtcgaatacctctggcggtgata | | |
| 1☆ | | BBa_I14015 | P(Las) TetO | ...ttttggtacactccctatcagtgatagaga | | |
| 1☆ | | BBa_I14016 | P(Las) CI0 | ...cttttggtacactacctctggcggtgata | | |
| 1☆ | | BBa_I14017 | P(Rhl) | ...tacgcaagaaaatggtttgttatagtcgaa | | |
| | | BBa_I739105 | Double Promoter (LuxR/HSL, positive / cI, negative) | ...cgtgccgtgttgtaaatacctggtgcctgttga | | |
| 1☆ | | BBa_I746104 | P2 promoter in agr operon from S. aureus | ...agattgtactaaatcgtataaatgatagtga | | |
| B | W | BBa_I751501 | plux-cI hybrid promoter | ...gtgttgatgctttatcacggccaggtgta | | |
| D | W | BBa_I751502 | plux-lac hybrid promoter | ...agtgtgtggaattgtgagcggataacaatt | | |
| A | | BBa_I761011 | CinR, CinL and glucose controlled promoter | ...acatcttaaaagttttgtatcatattcgt | | |
| | | BBa_J06403 | RhlR promoter repressible by CI | ...tacgcaagaaaatggtttgttatagtcgaa | | |
| | | BBa_J102001 | Reverse Lux Promoter | ...tcttgcgtaaaacttgtacgatcctacaggt | | |
| | | BBa_J64000 | rhlI promoter | ...atcctcctttagtctcccctccatgtgtg | | |
| | | BBa_J64010 | lasI promoter | ...taaaattatgaaattgcataaatcctca | | |
| B | | BBa_J64057 | LuxR-3OC6HSL independent R0065 | ...gtgttgactatttacctctggcggtgata | | |
| | | BBa_J64712 | LasR/LasI inducible & RhlR/RhlI repressible Promoter | ...gaaatcggcagttttggtcacgaaagc | | |
| 1☆ | | BBa_K091107 | pLux/cI Hybrid Promoter | ...acacccgtgcgtgttgatatagtcgaataaa | | |
| A | | BBa_K091117 | pLas promoter | ...aaaattatgaaattgtataaatcttcag | | |
| 1☆ | | BBa_K091143 | pLas/cI Hybrid Promoter | ...ggttcttttgtgtacctctggcggtgataa | | |
| 1☆ | | BBa_K091146 | pLas/Lux Hybrid Promoter | ...tgtaggatcgtacagtatataaatcttcag | | |
| B | W | BBa_K091156 | pLux | ...caagaaaaatggtttgttatagtcgaataaa | | |
| B | | BBa_K091157 | pLux/Las Hybrid Promoter | ...ctatccattgtctagtatagtcgaataaa | | |
| 1☆ | | BBa_K145150 | Hybrid promoter: HSL-LuxR activated, P22 C2 repressed | ...taattatcaattaagttgttcttaattcc | | |
| S | W | BBa_K265000 | PAI-LasR -> LuxI (AI) | ...caccttcgggtgggcctttctgcgtttata | | |
| S | W | BBa_K265005 | PAI-LasR -> LasI & AHL-LuxR --\| LasI | ...aataactctgatagtgctagtgtagatctc | | |
| S | W | BBa_K265006 | PAI-LasR -> LasI-GFP & AI-LuxR --\| LasI-GFP | ...caccttcgggtgggcctttctgcgtttata | | |
| S | W | BBa_K265007 | Complex QS -> LuxI & LasI circuit | ...caccttcgggtgggcctttctgcgtttata | | |
| 1☆ | | BBa_R0061 | Promoter (HSL-mediated luxR repressor) | tgacacctgtaggatcgtacaggtataat | | |
| 1☆ | | BBa_R0062 | Promoter (luxR & HSL regulated -- lux pR) | ...caagaaaatggtttgttatagtcgaataaa | | |
| 1☆ | | BBa_R0063 | Promoter (luxR & HSL regulated -- lux pL) | ...cacgcaaaactgcgacaaacaattaggtaa | | |
| 1☆ | | BBa_R0071 | Promoter (RhlR & C4-HSL regulated) | ...gttagcttcgaattggctaaaaagtgtc | | |
| 1☆ | | BBa_R0078 | Promoter (cinR and HSL regulated) | ...ccatctgcttccacgaacttgaaaacgc | | |
| 1☆ | | BBa_R0079 | Promoter (LasR & PAI regulated) | ...ggcgcgggtctttttggtcacgaaagc | | |
| 1☆ | | BBa_R1062 | Promoter, Standard (luxR and HSL regulated -- lux pR) | ...aagaaaatggtttgttatagtcgaataaa | | |

- And the Inputs are ...
- Smart systems cue themselves; human-proofing

# Controlling the system

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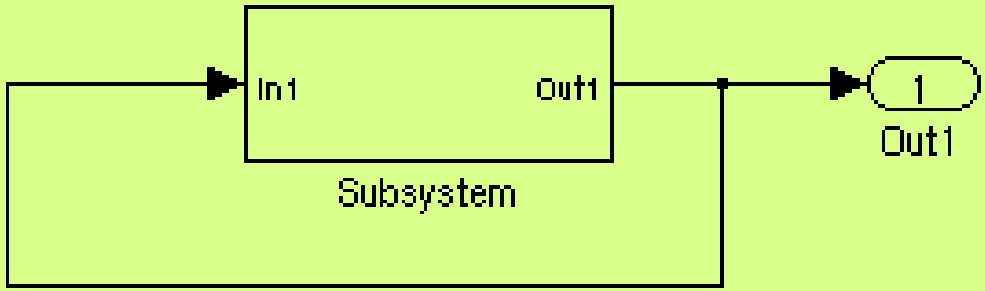
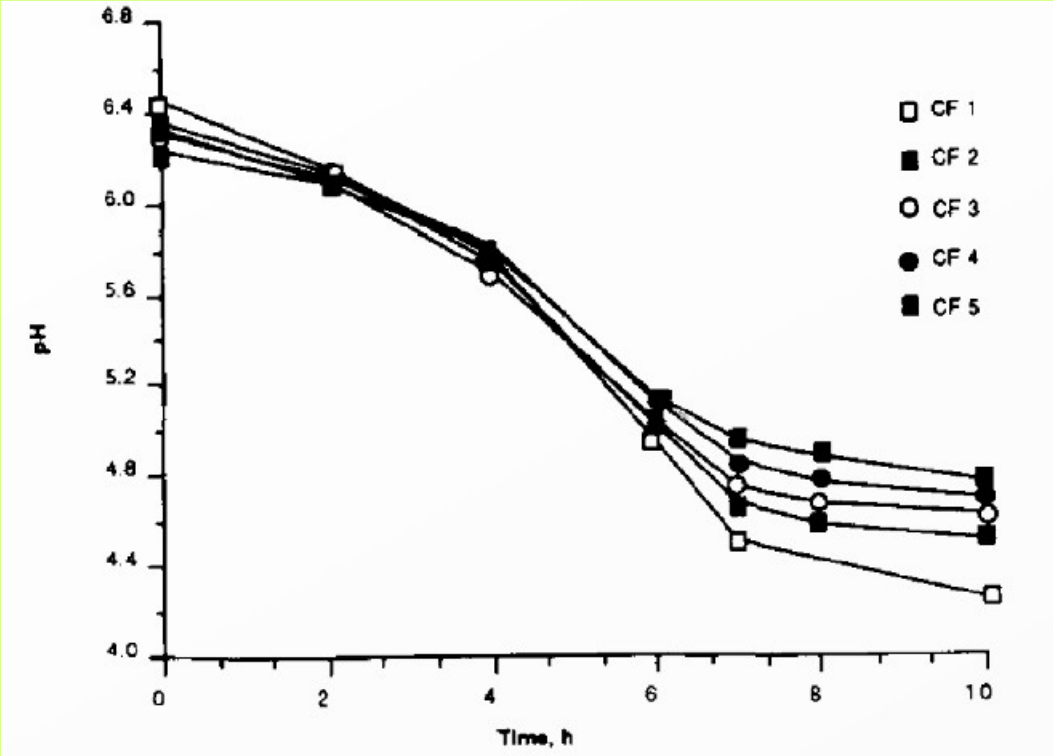
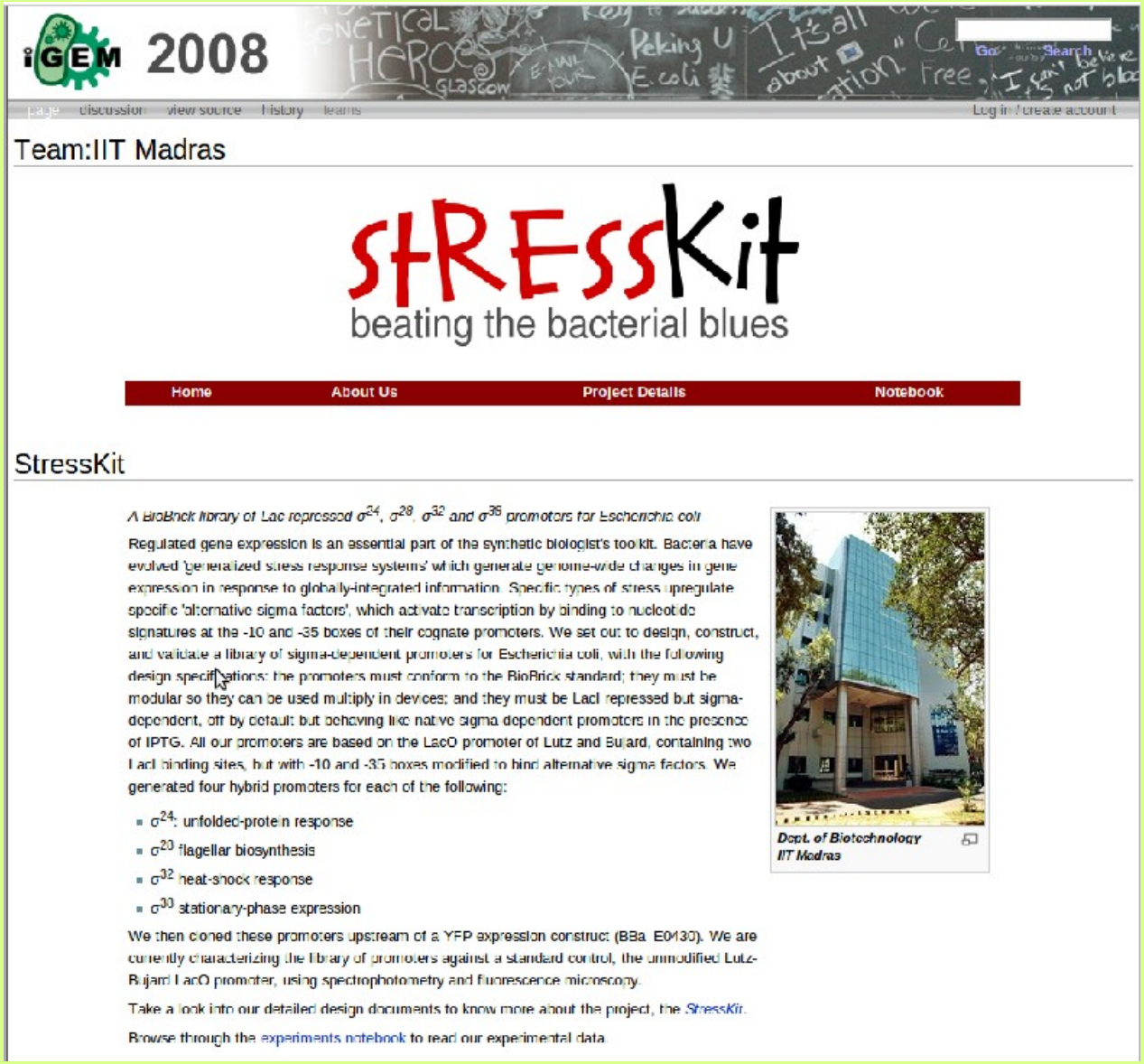


# Controlling the system

- Curdling
  - Heat shock
  - pH
  - Externally inducible (Nisin)
- Non-leaky, good response, system based.



# Combine and conquer



iGEM 2008

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Team:IIT Madras

stRESsKit

beating the bacterial blues

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StressKit

*A BioBrick library of Lac repressed $\sigma^{24}$, $\sigma^{28}$, $\sigma^{32}$ and $\sigma^{36}$ promoters for Escherichia coli*

Regulated gene expression is an essential part of the synthetic biologist's toolkit. Bacteria have evolved 'generalized stress response systems' which generate genome-wide changes in gene expression in response to globally-integrated information. Specific types of stress upregulate specific 'alternative sigma factors', which activate transcription by binding to nucleotide signatures at the -10 and -35 boxes of their cognate promoters. We set out to design, construct, and validate a library of sigma-dependent promoters for Escherichia coli, with the following design specifications: the promoters must conform to the BioBrick standard; they must be modular so they can be used multiply in devices; and they must be LacI repressed but sigma-dependent, off by default but behaving like native sigma dependent promoters in the presence of IPTG. All our promoters are based on the LacO promoter of Lutz and Bujard, containing two LacI binding sites, but with -10 and -35 boxes modified to bind alternative sigma factors. We generated four hybrid promoters for each of the following:

- $\sigma^{24}$: unfolded-protein response
- $\sigma^{28}$ flagellar biosynthesis
- $\sigma^{32}$ heat-shock response
- $\sigma^{36}$ stationary-phase expression

We then cloned these promoters upstream of a YFP expression construct (BBa_E0130). We are currently characterizing the library of promoters against a standard control, the unmodified Lutz-Bujard LacO promoter, using spectrophotometry and fluorescence microscopy.

Take a look into our detailed design documents to know more about the project, the StressKit.

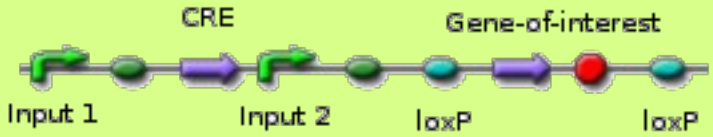
Browse through the experiments notebook to read our experimental data.

Dept. of Biotechnology
IIT Madras

- Fusion of promoters / stress factors?
- Statistical chance for the right combination

# Recombinase to the rescue

- The CRE floxes DNA between loxP sites.
- Oreientation-dependent splicing
- Knockout, the biological AND.

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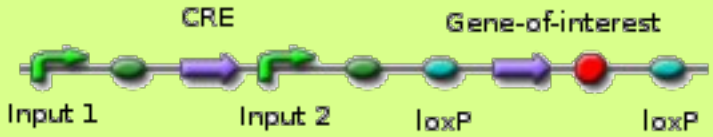


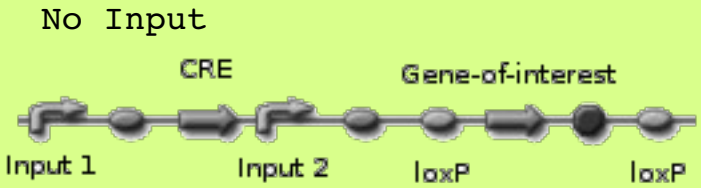

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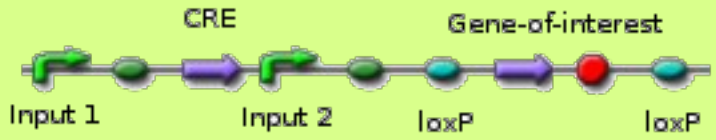



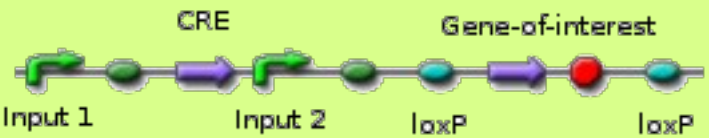

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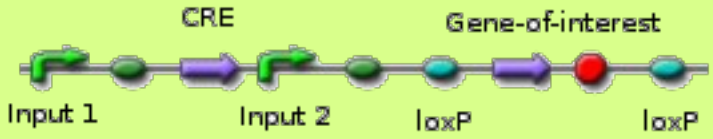



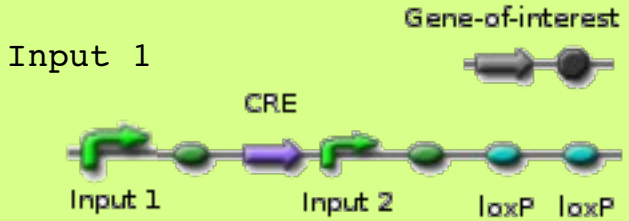

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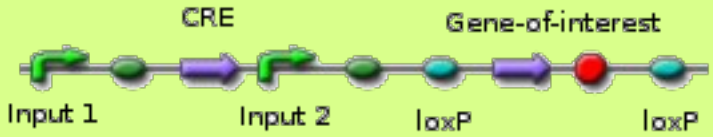



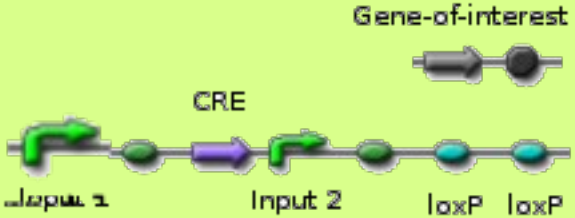


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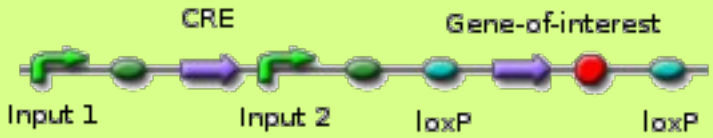



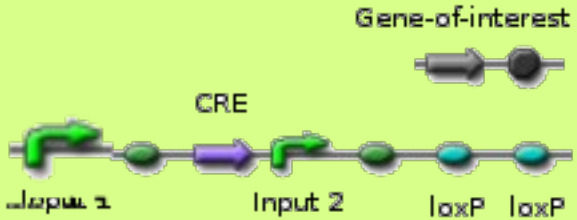


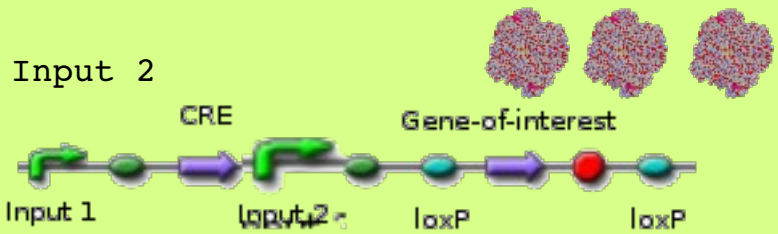

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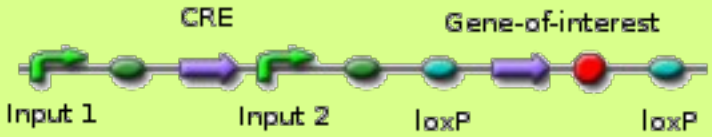


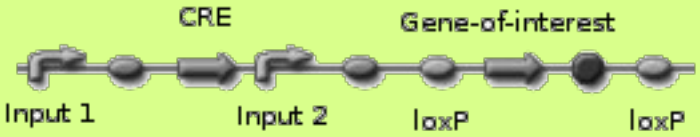


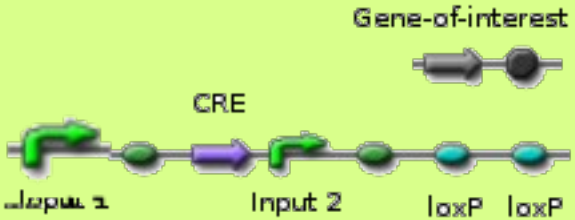


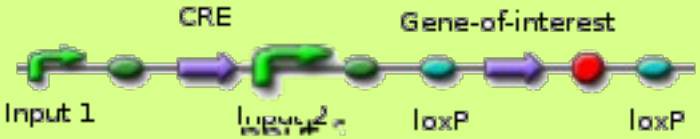


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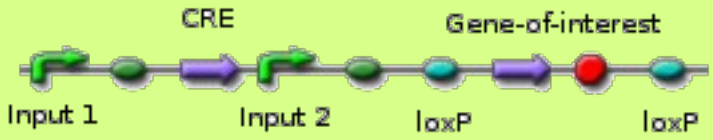
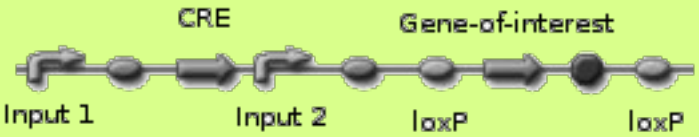
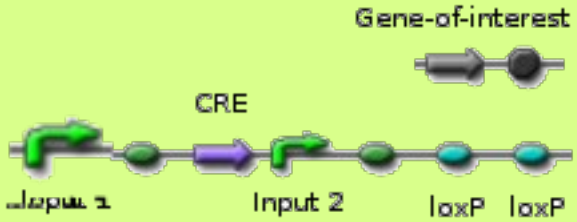
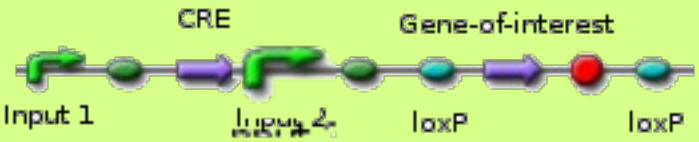
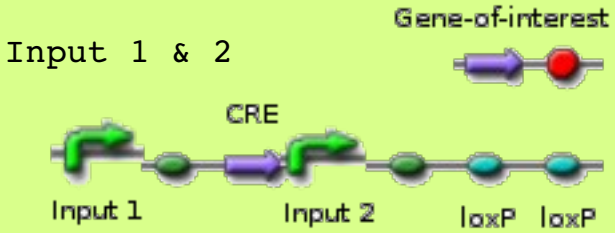


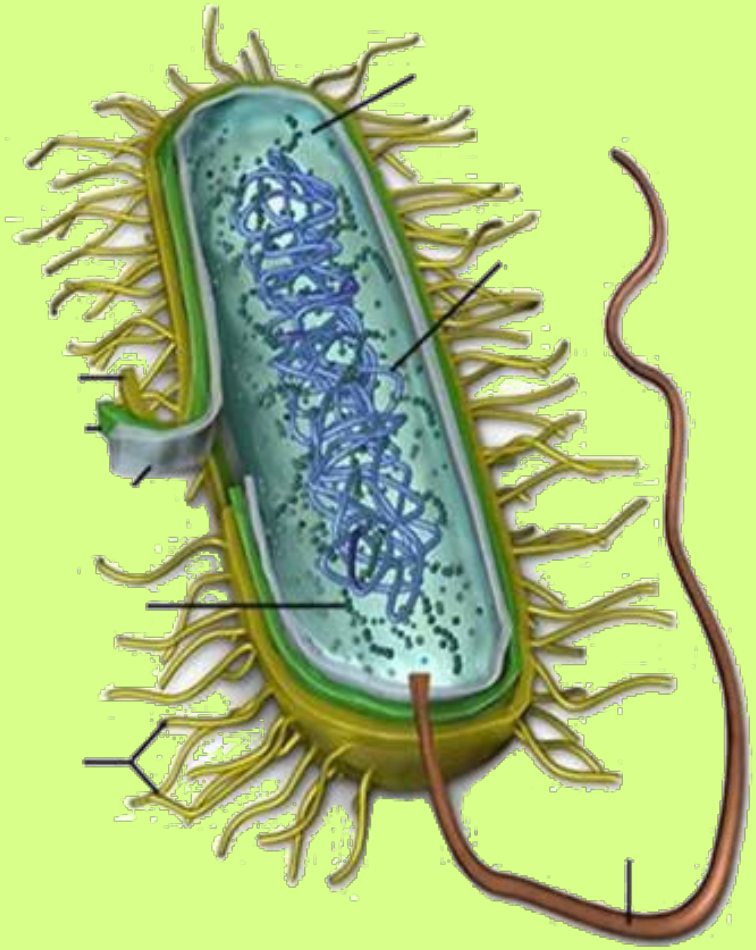



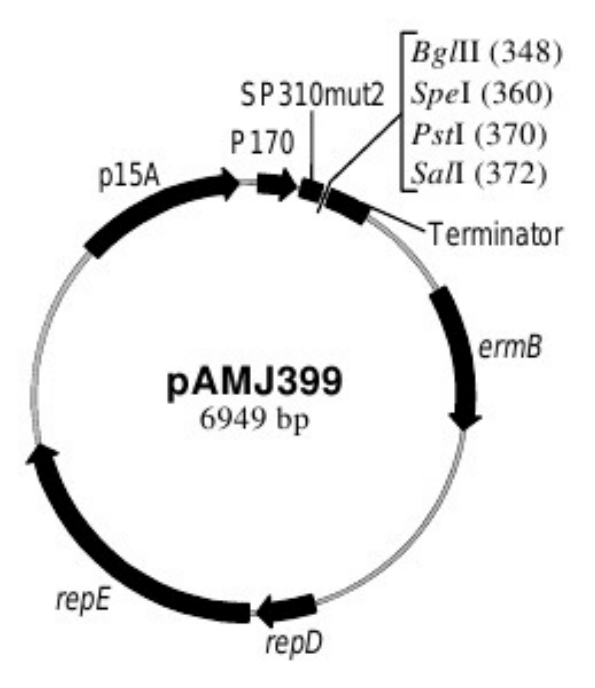
# Cellular Chasis

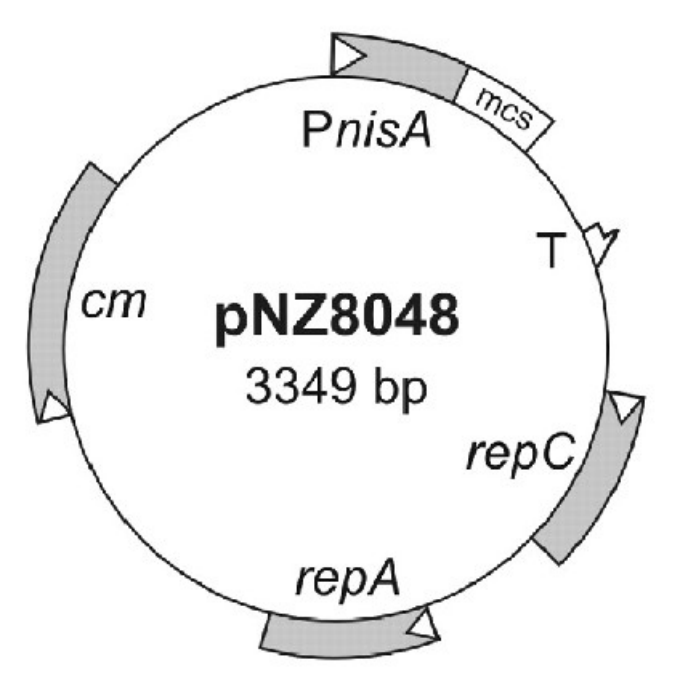


- The model organism
- *E.coli* vs *L.lactis*
- Final application in LAB.

# Promoters

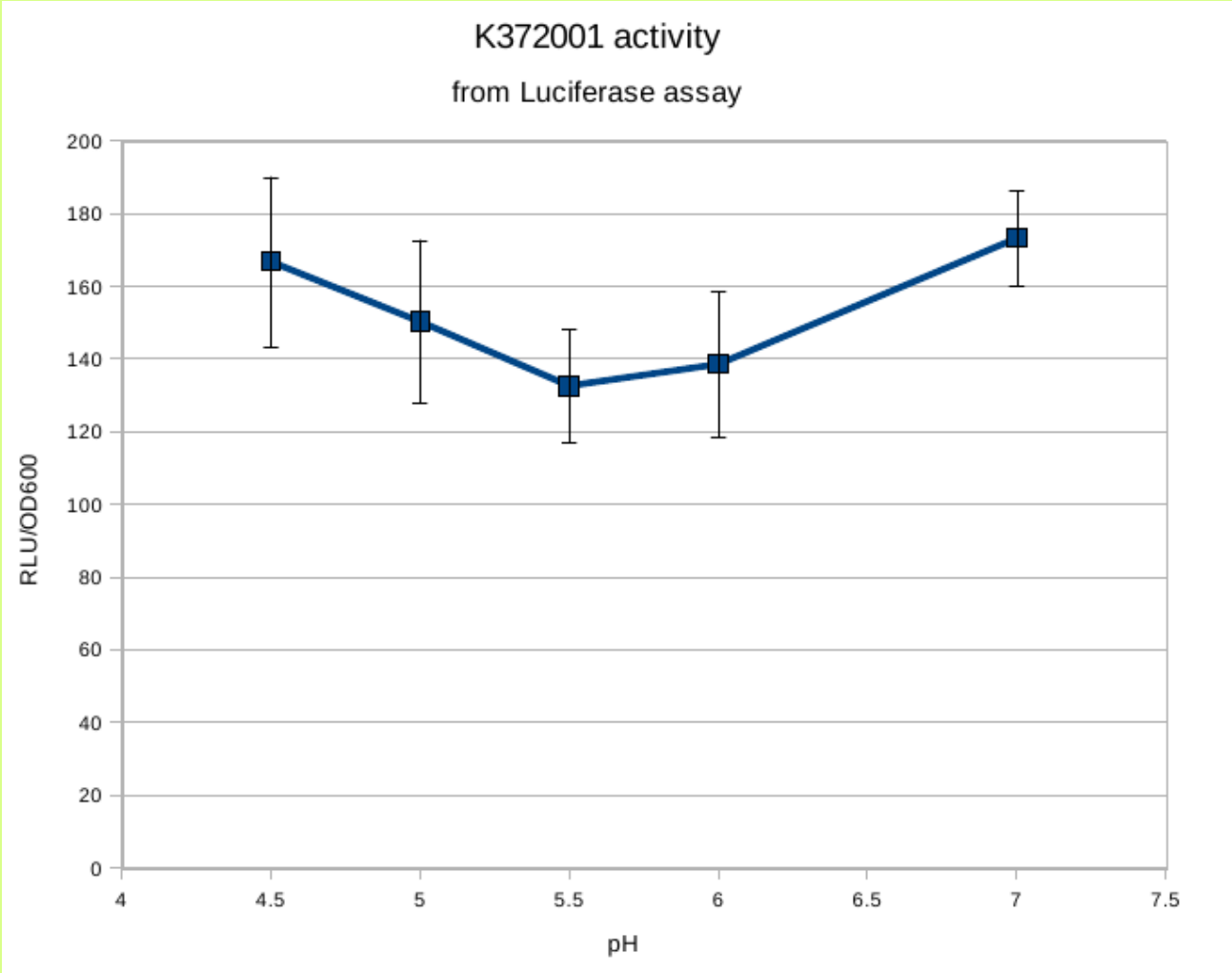
- pH and external induction.
- Window of expression to be characterised
- Obtained by PCR extraction



# Promoters

- Upregulation at mildly acidic pH.
- Serendipity



# Promoters

- Upregulation at mildly acidic pH.
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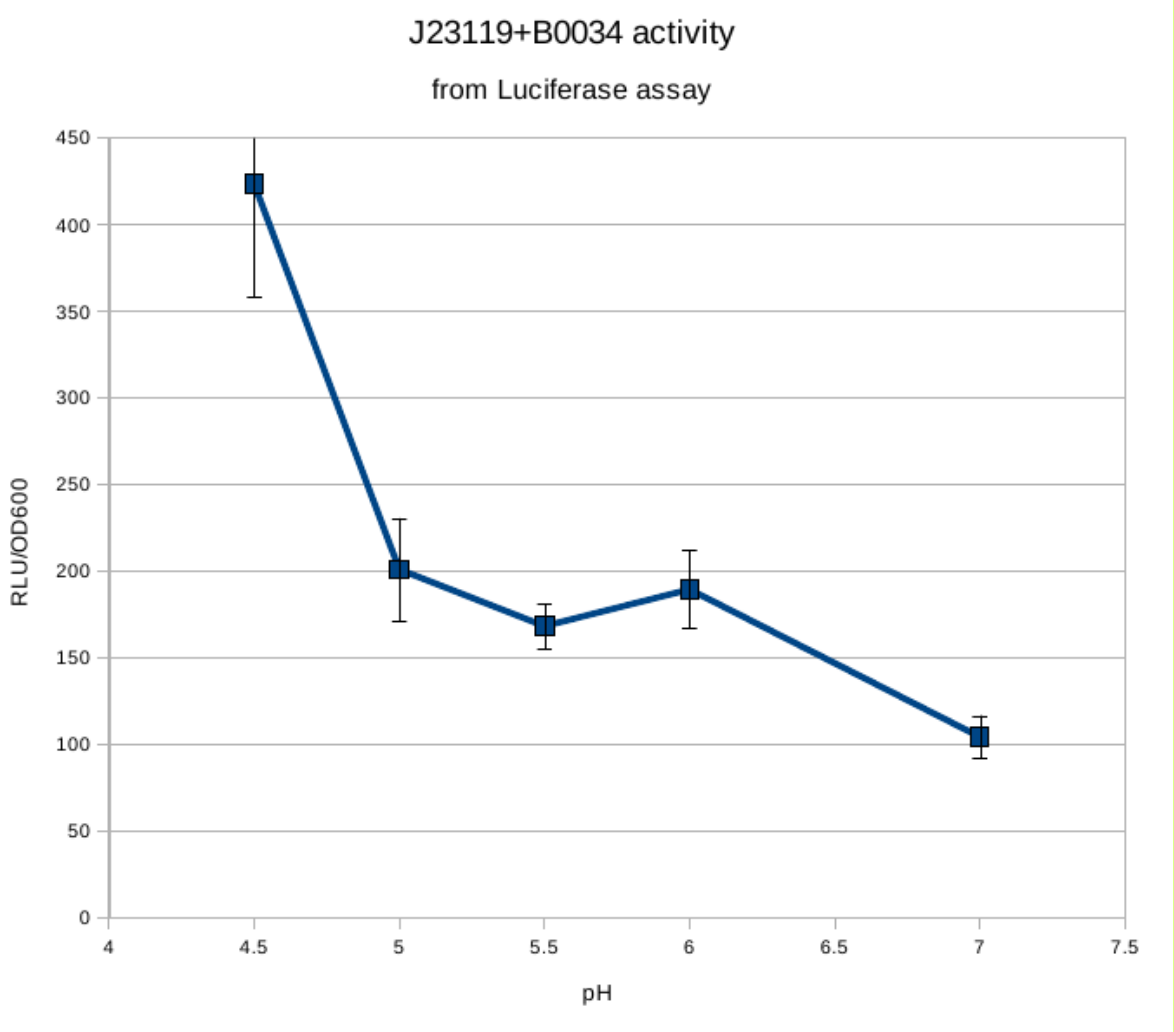
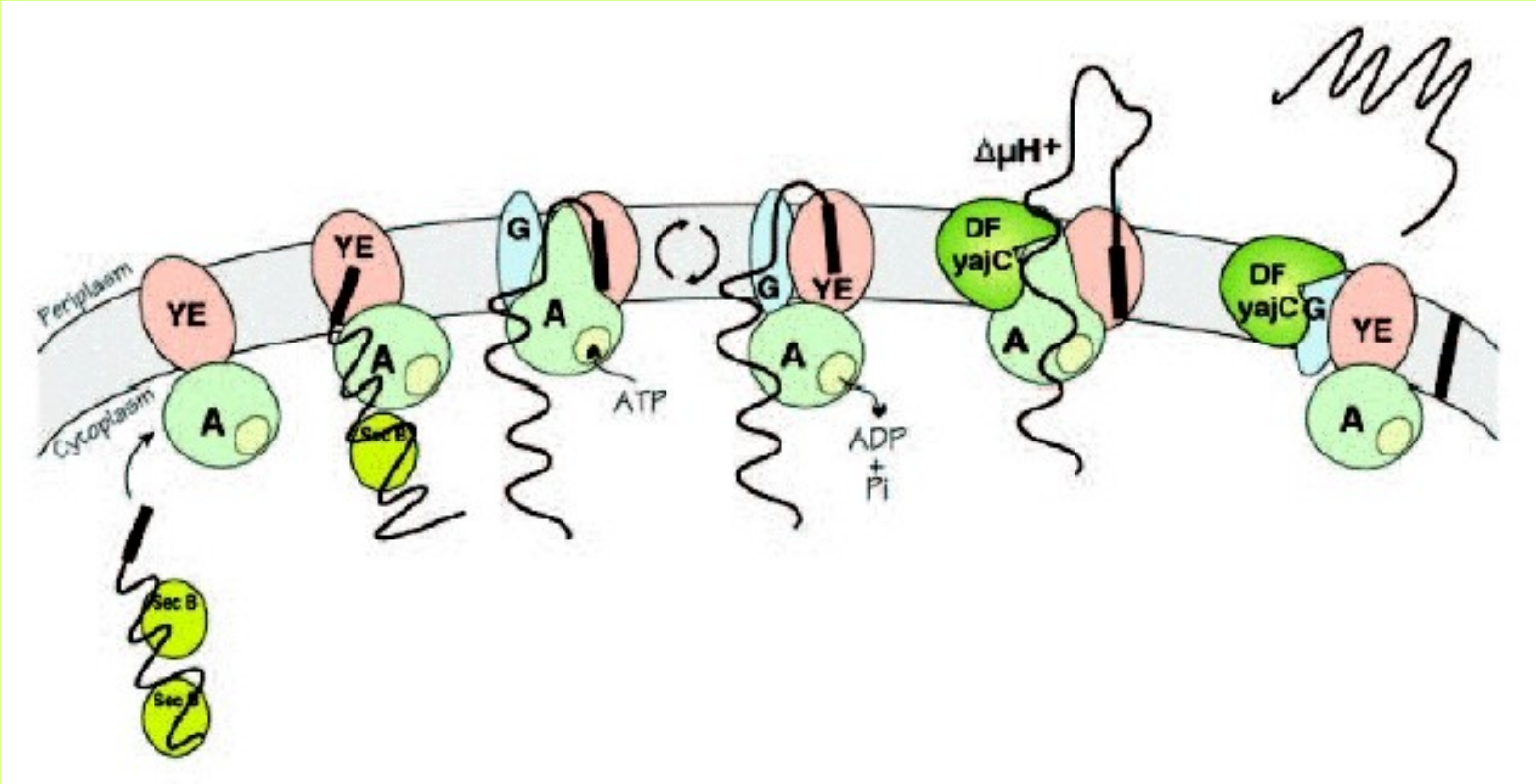


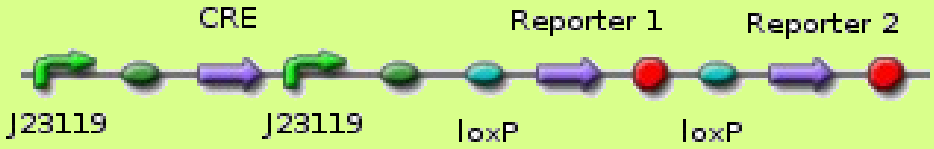
# Export

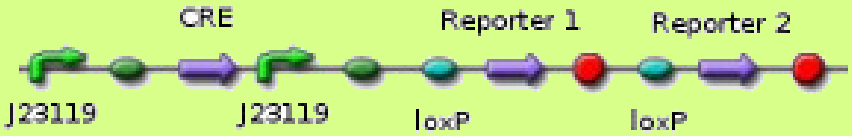
- Expelling the protein from the cell
- Luck of the draw
- Optimisation



# The pseudo-AND Gate

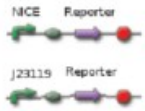
- Appropriate placement of loxP
- Excision of inserted DNA.



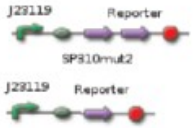




# Final Construct

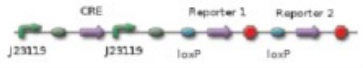
**NICE**
The Nisin-inducible expression system is upregulated under the prescence of food-grade nisin in the system. It is also to be noted that the system should only be activated after the culture reaches an OD600 of around 0.7. The system is tested with a reporter with J23119 as a reference.



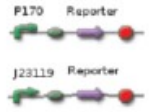

**SP310mut2**
The export tag is that which is obtained from the same vector as P170 and is an optimised version of the naturally found SP310. The system is tested under J23119 with a reporter with the same reporter without the export tag as a reference.

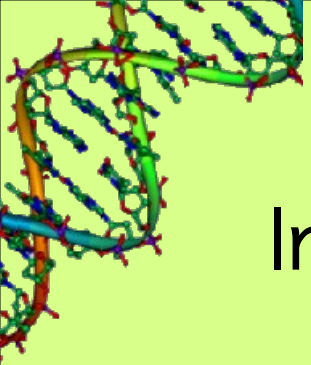



**CRE-loxP**
The CRE enzyme is a recombinase that modifies / excises the DNA fragment that lies between its specific recognition sites, named lox. We have chosen to test the system with two reporters, and the promoter J23119.




**P170**
This is a pH-sensitive growth phase-dependent promoter that upregulates expression from a baseline between a pH of 6.0 and 6.5. The system is to be tested with a reporter with J23119 as a reference.





# In Conclusion,

- We designed an implementation of a single-use, AND logic in DNA.
- Applied the mechanism to concieve a sweetening protein using inputs from the chemistry of the process.
- Identified two promoters, and an export tag for the system, submitted one promoter and the export tag and characterised the acid tolerance promoter, P170.
- Presented a novel approach to GM, where the system is excised after complete expression.

# Our team

- Advisors :
  - Dr Chandraraj K
  - Dr Guhan Jayaraman
- Instructors :
  - Shashi Bala Prasad
  - Parashuraman

## Our Sponsors

DR. REDDY'S

INDIAN INSTITUTE OF TECHNOLOGY MADRAS
Industrial Consultancy & Sponsored Research - IIT Madras

NEW ENGLAND BioLabs Inc.

Labmate (Asia)

# References


- Morris J A, et al; Purification of monellin, the sweet principle of D.cumminsii, Biochem Biophys Acta (1972), vol 261:114
- Madsen S M, et al; Molecular characterisation of the pH-inducible and growth phase-dependent promoter P170 of L.lactis; Molecular Microbiology (1999), vol 32, pg 75 - 87
- Ravn P, et al; Optimisation of signal peptide SP310 for heterologous protein production in L.lactis; Microbiology (2003), vol 149, pg 2193 - 2201
- Pfeifer A, et al; Delivery of the Cre recombinase by a self-deleting lentiviral vector: efficient gene targetting in vivo.
- Srilaorkul S, et al; Growth and activity of *L.lactis ssp. Cremoris* in ultrafiltered skim milk, Journal of Diary Science (1989), vol 72, pg 2435 - 2443
- http://partsregistry.org, http://2008.igem.org/Team:IIT_Madras, last accessed on 07 Nov 2010 at 0550 (ECT).
- http://www.thehindu.com/news/national/article82528.ece, http://beta.thehindu.com/news/cities/Delhi/article97706.ece, http://click4biology.info/c4b/7/images/7.5/catalase.gif & http://www.ncbi.nlm.nih.gov/bookshelf/picrender.fcgi?book=eurekah&part=A41672&blobname=ch267f1.jpg last accessed on 07 Nov 2010 at 0550 (ECT).