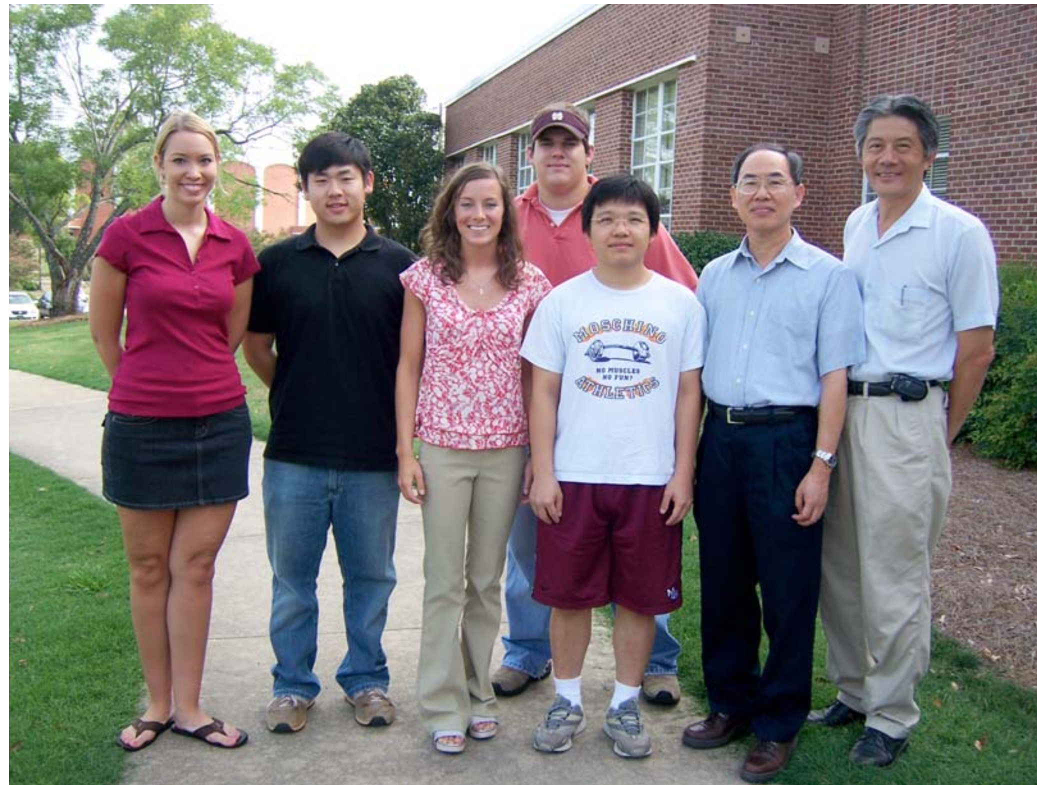
Genetically Engineered H_2 Detector

Mississippi State University

- Departmental Goal – Produce oil for biodiesel
- Dr. To wanted to find out if we could engineer bacteria to produce biodiesel efficiently
- We decided to start small

- Learn procedure for genetically engineered machines
- Combine knowledge from several departments
  - ABE, BCH, ChE, ECE
- Develop network with MIT and other iGEM partners to allow for future collaboration

Our Team



- Agricultural and Biological Engineering
  - Dr. Filip To
- Biochemistry
  - Dr. Din-Pow Ma
- Electrical and Computer Engineering
  - Dr. Bob Reese
- Chemical Engineering
  - Dr. Todd French

Our Team - Students

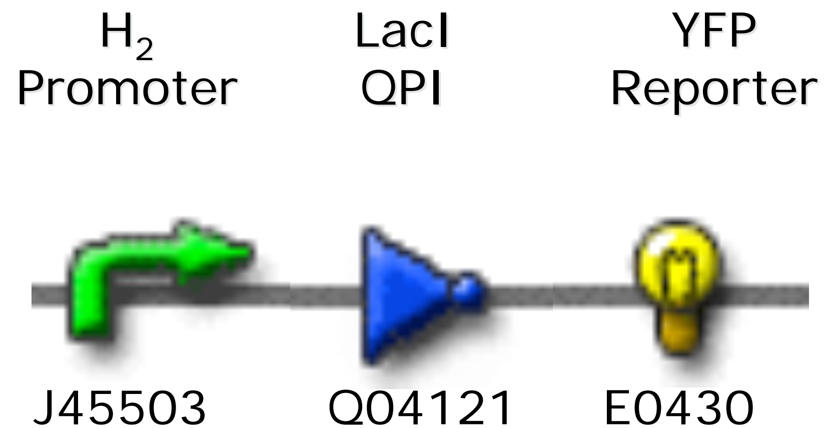
- Agricultural and Biological Engineering
  - Graduate
    - Brendan Flynn, Robert Morris
  - Undergraduate
    - Teri Vaughn, Lauren Beatty, Scott Tran, Joe Chen, Sam Pote, Paul Kimbrough
- Biochemistry
  - Graduate
    - Victor Ho

- We wanted to design a machine to detect the presence of H_2
- The machine would function when “turned on” by an inducer
- The machine would produce quantifiable fluorescence dependent on H_2 concentration

- Quantifiable detection of H_2
- This function could possibly be incorporated into bacteria used in the production of H_2 in the future

Design - Parts

Our Composite Part - J43001



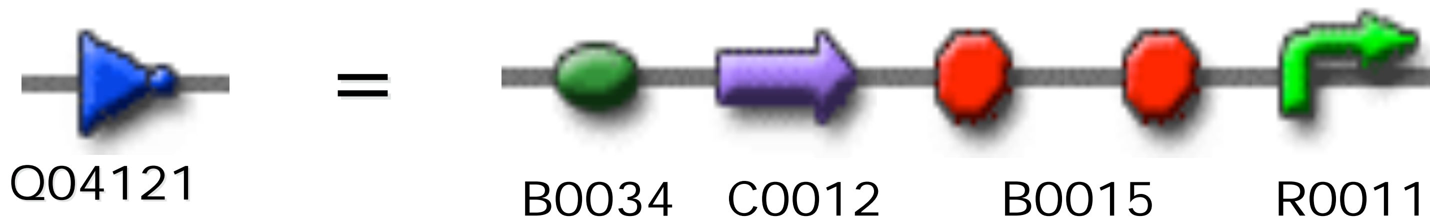
Design – Part Descriptions

J45503	Cold shock promoter and H_2 promoter
Q04121	LacI QPI (Quad Part Inverter), composite
E0430	YFP output device, composite

J45503 Q04121 E0430

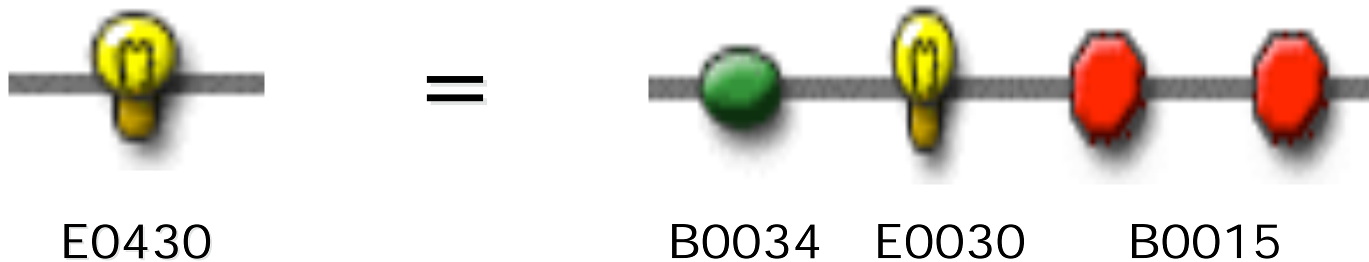
Design – Subparts – Q04121

Subpart	Description
B0034	Strong RBS
C0012	LacI coding region
B0015	Double terminator
R0011	Strong promoter, Repressed by LacI, Induced by IPTG

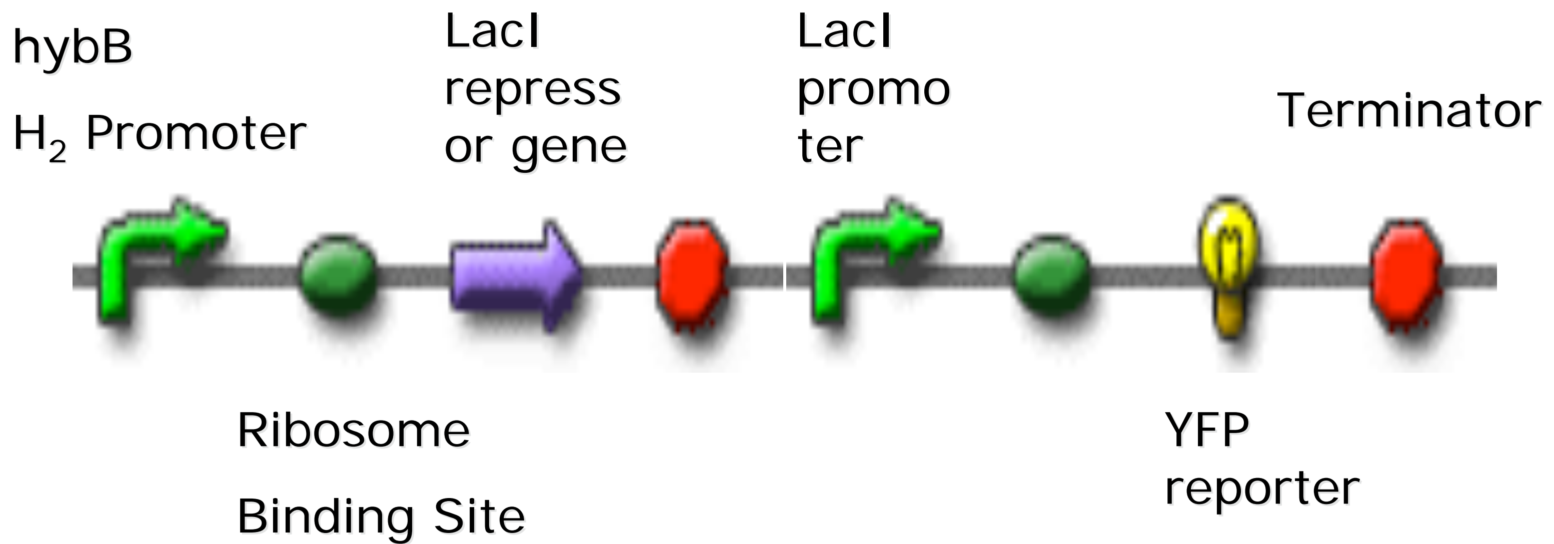


Design – Subparts – E0430

Subpart	Description
B0034	Strong RBS
E0030	YFP coding
B0015	Double terminator

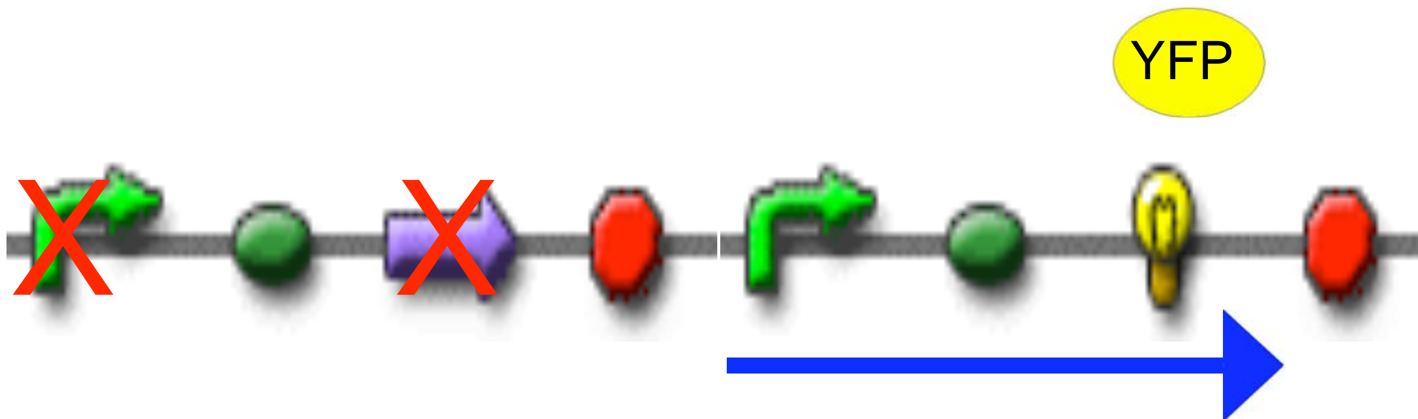


Design - Components



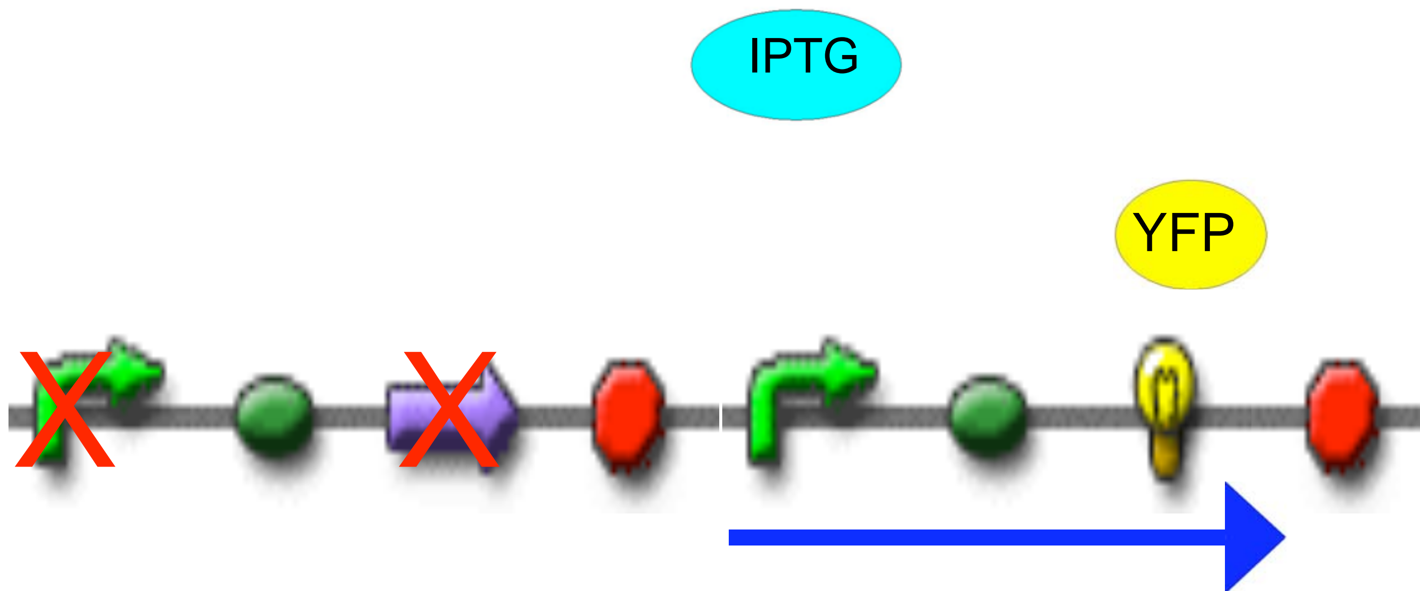
J43000 In Action

Lac promoter naturally shows an active but low transcription level



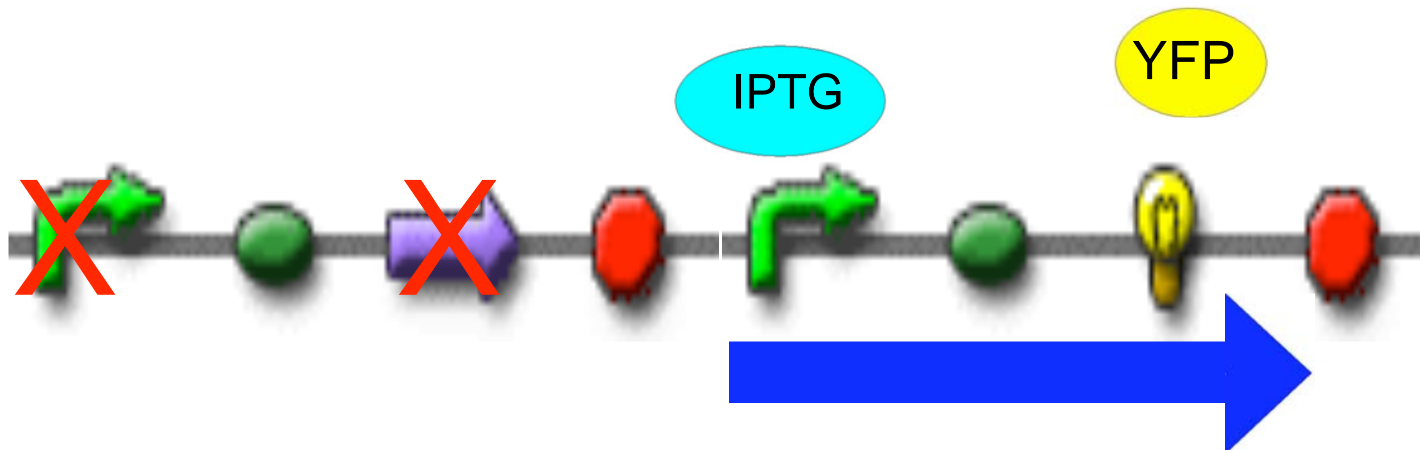
J43000 In Action

IPTG is a synthetic inducer



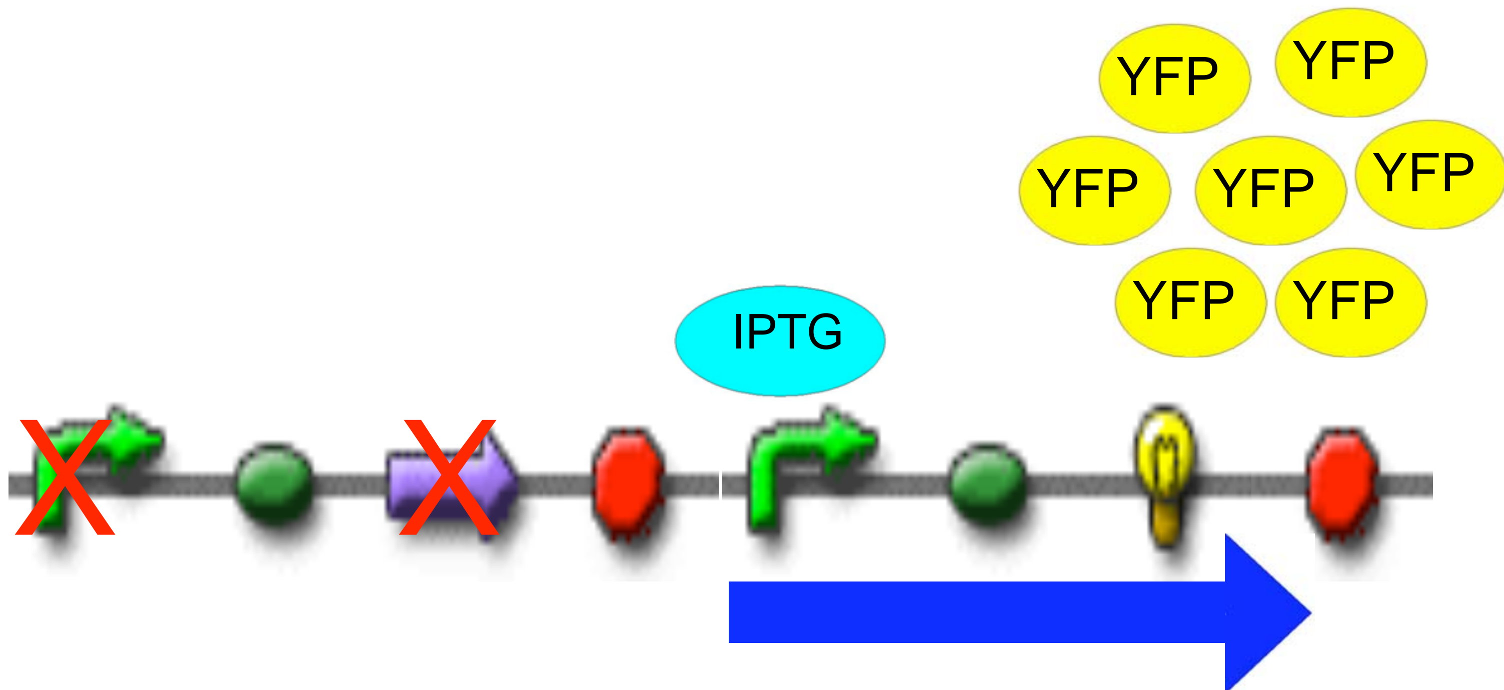
J43000 In Action

IPTG induces a high level of transcription at the Lac Promoter



J43000 In Action

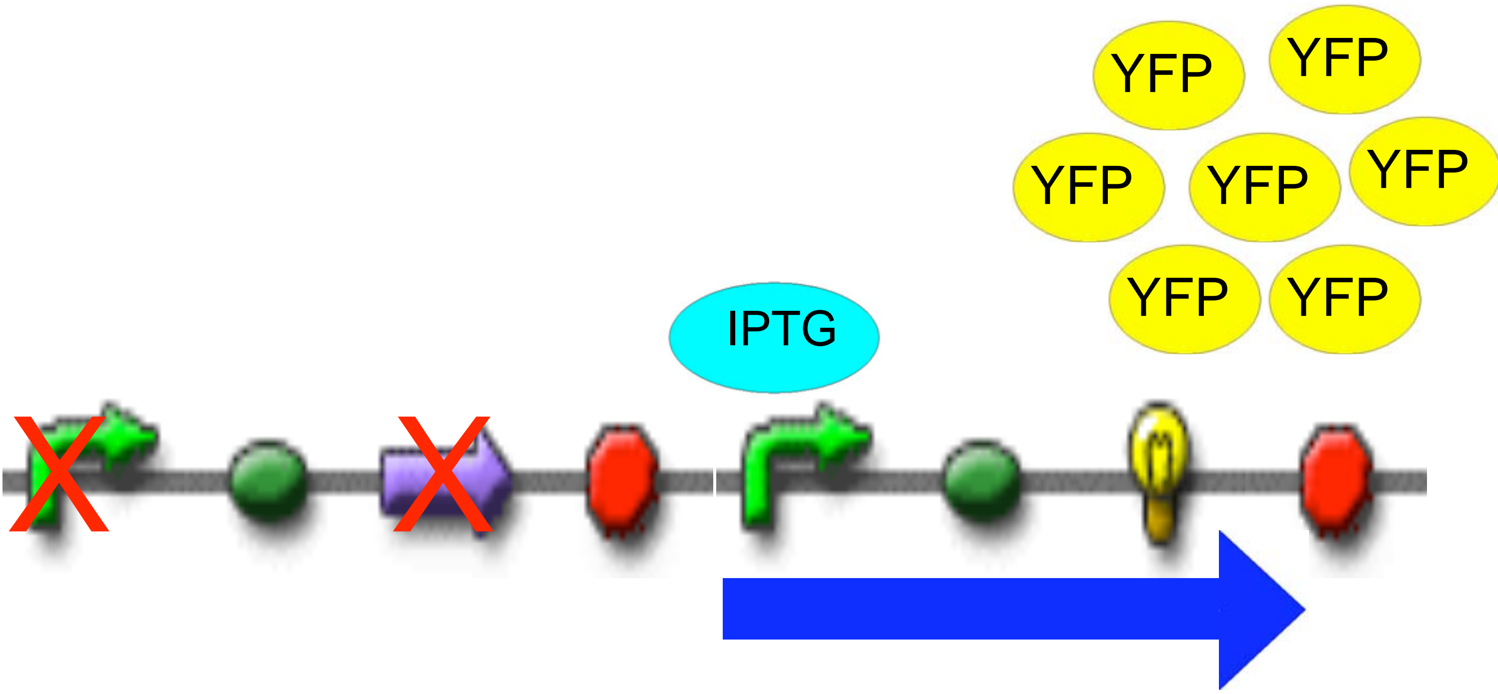
A large amount of YFP is transcribed and translated



J43000 In Action

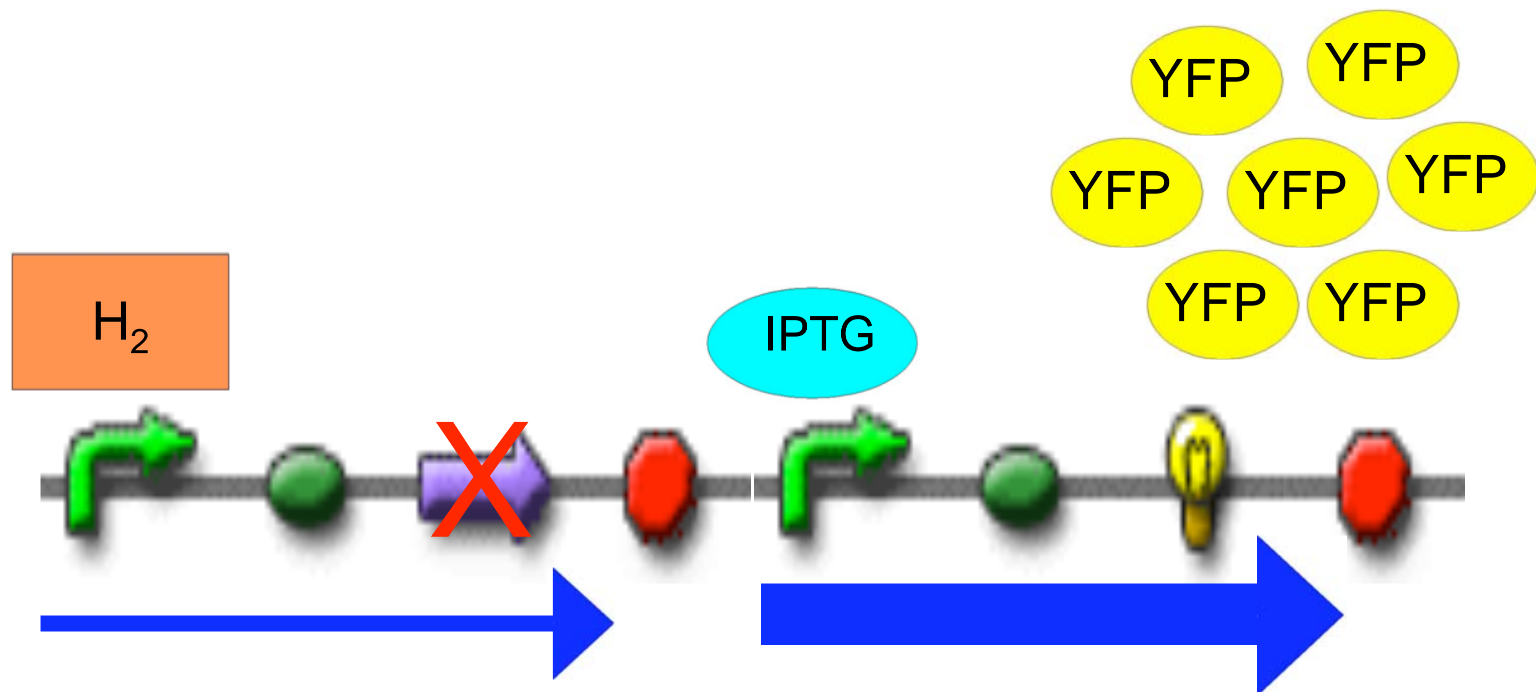
H_2

Hydrogen is introduced to the machine



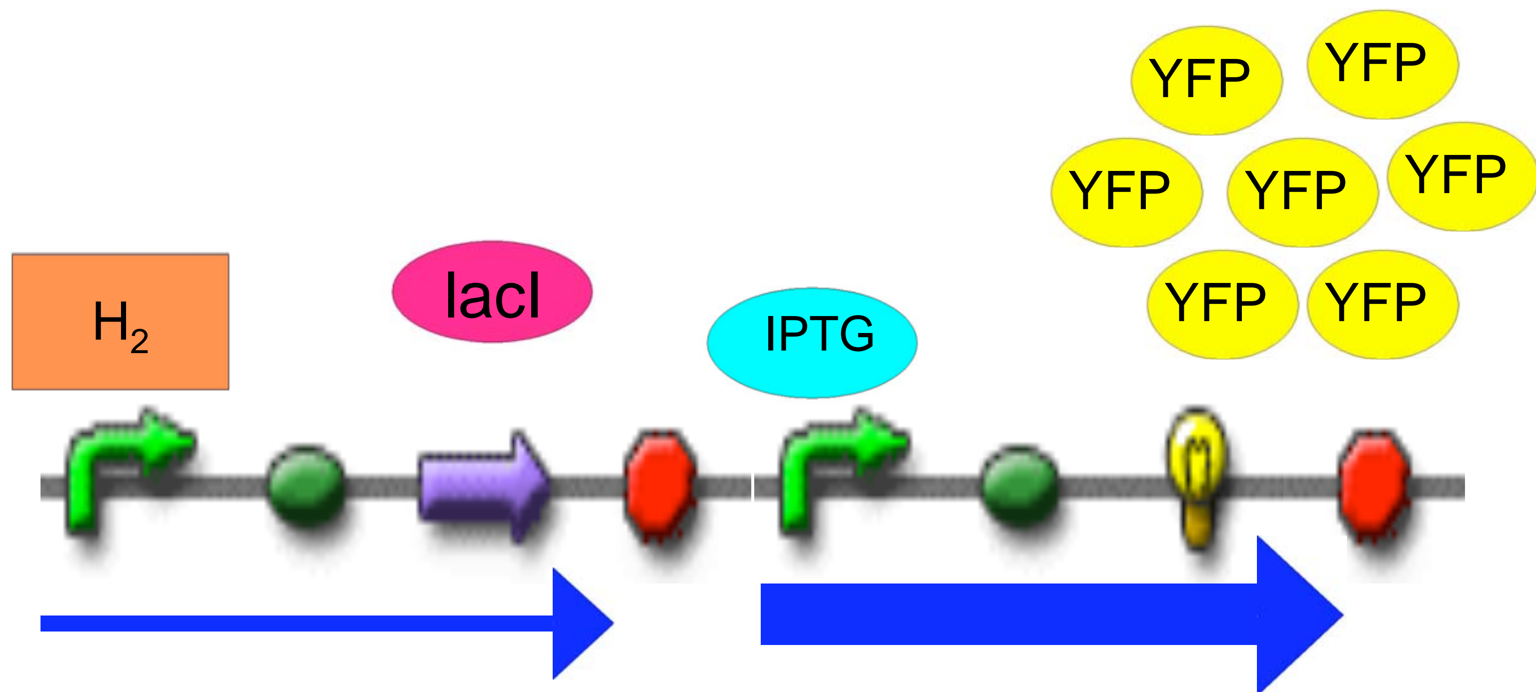
J43000 In Action

Hydrogen turns on the hybB promoter



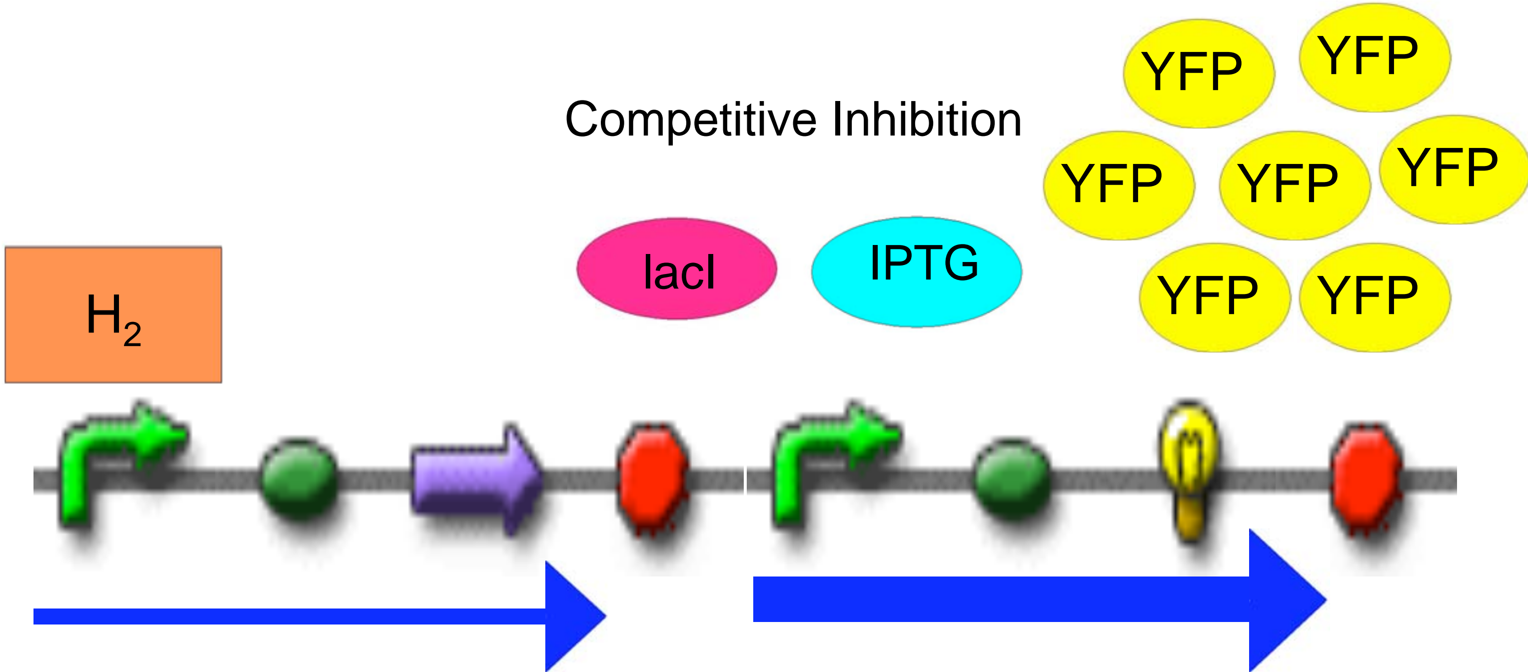
J43000 In Action

The lacI gene is transcribed



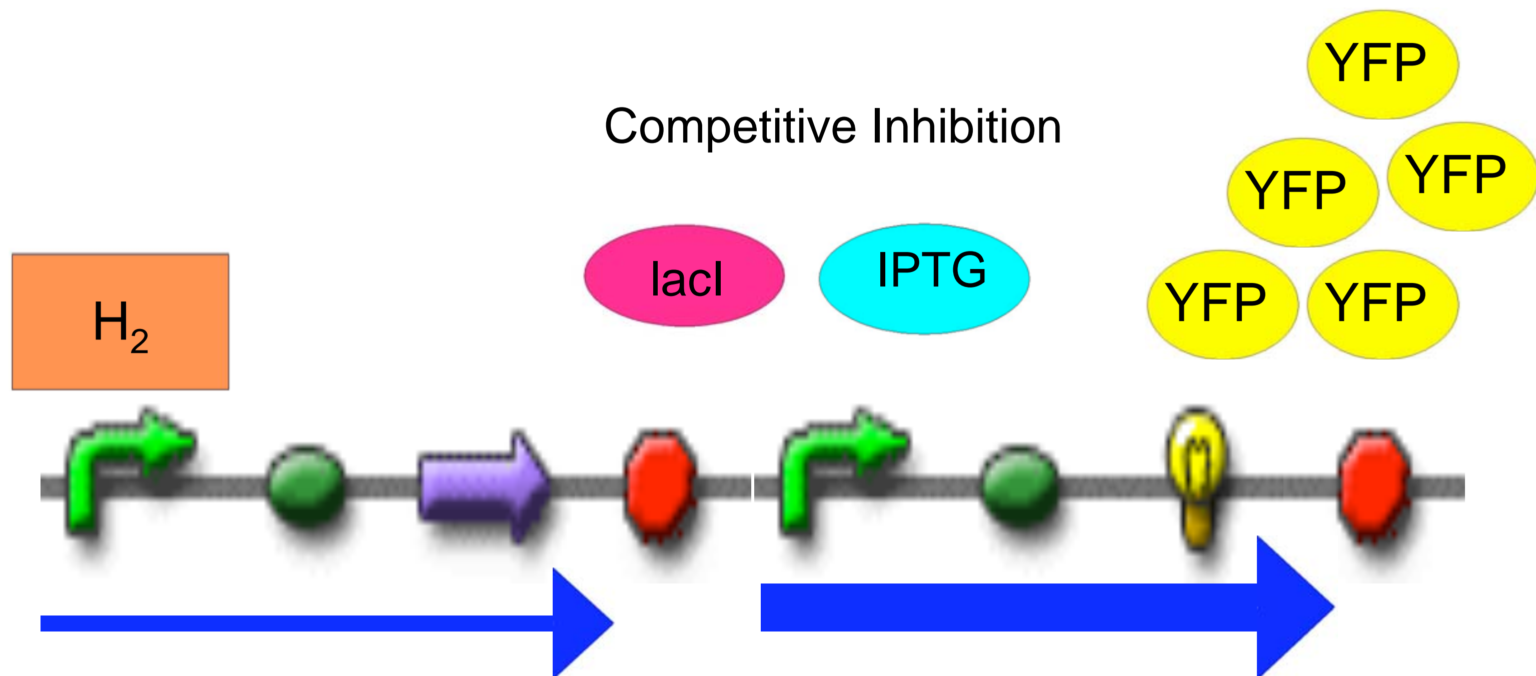
J43000 In Action

LacI repressor inhibits the lac promoter



J43000 In Action

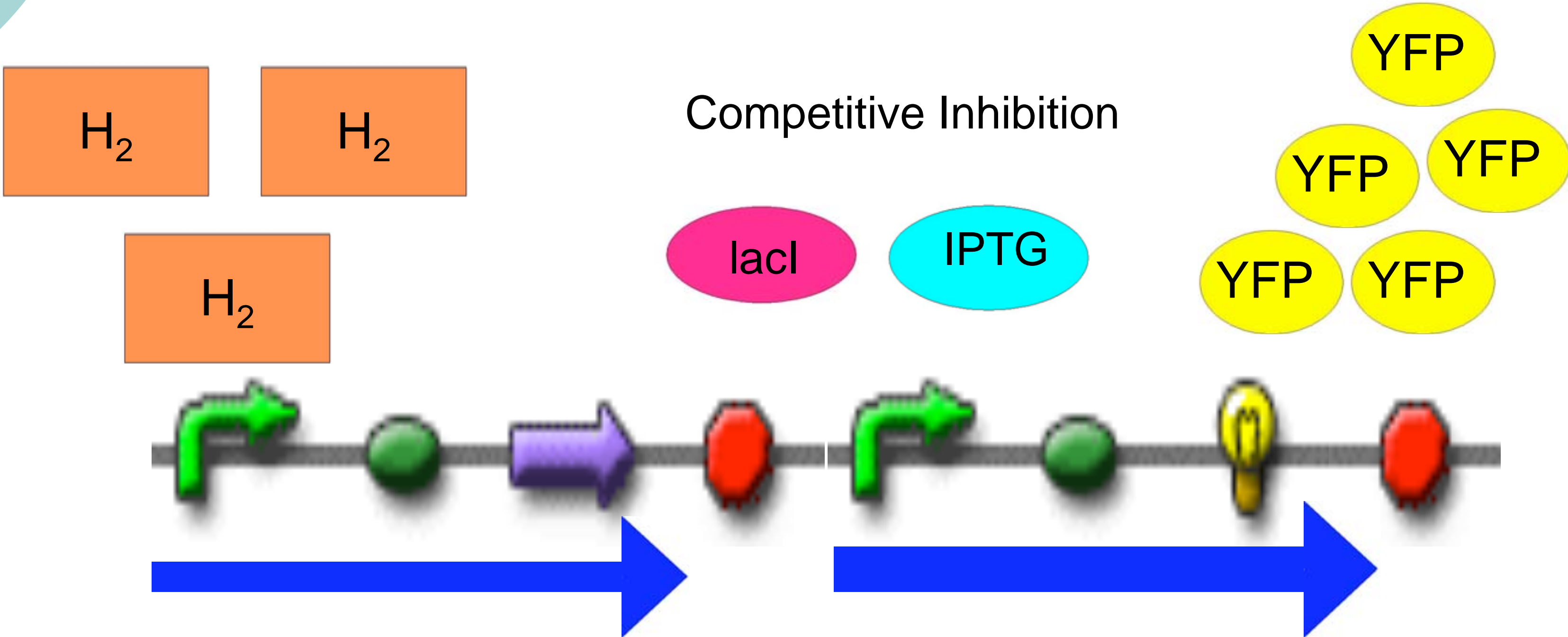
Lac promoter transcription is reduced, leading to lower YFP transcription



J43000 In Action

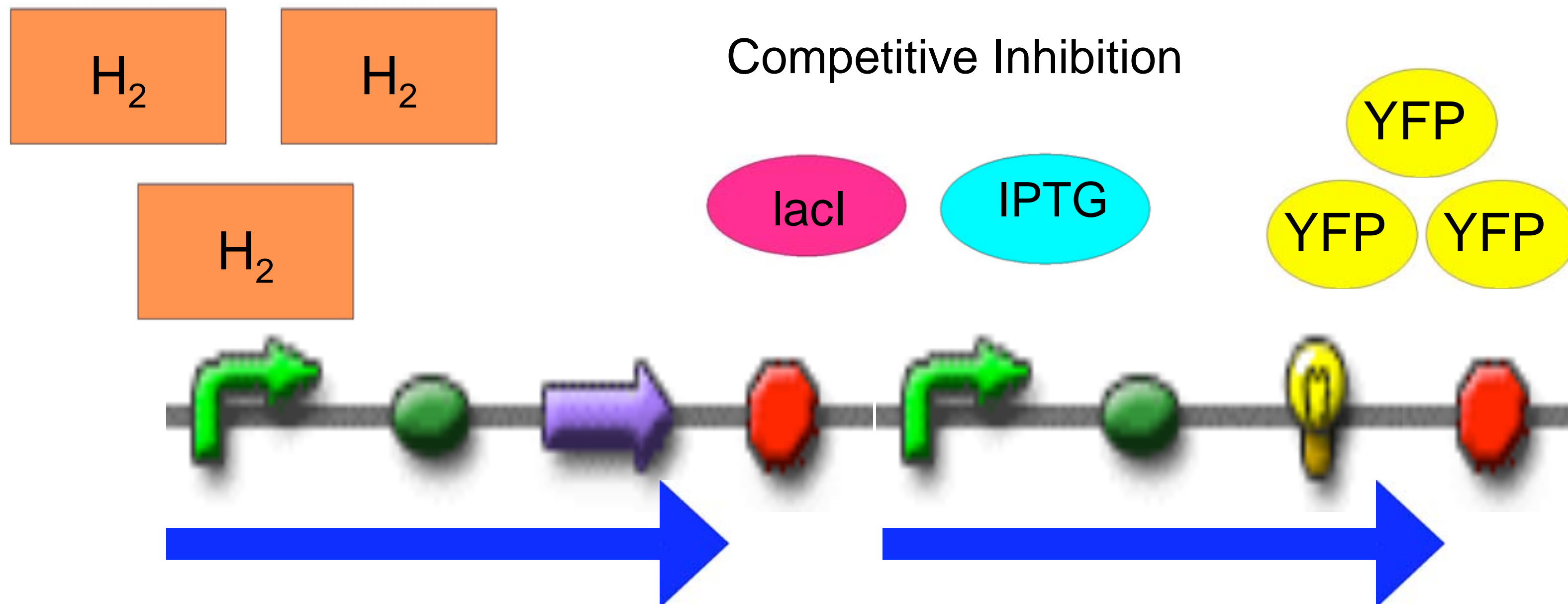
Hydrogen concentration is increased

Competitive Inhibition



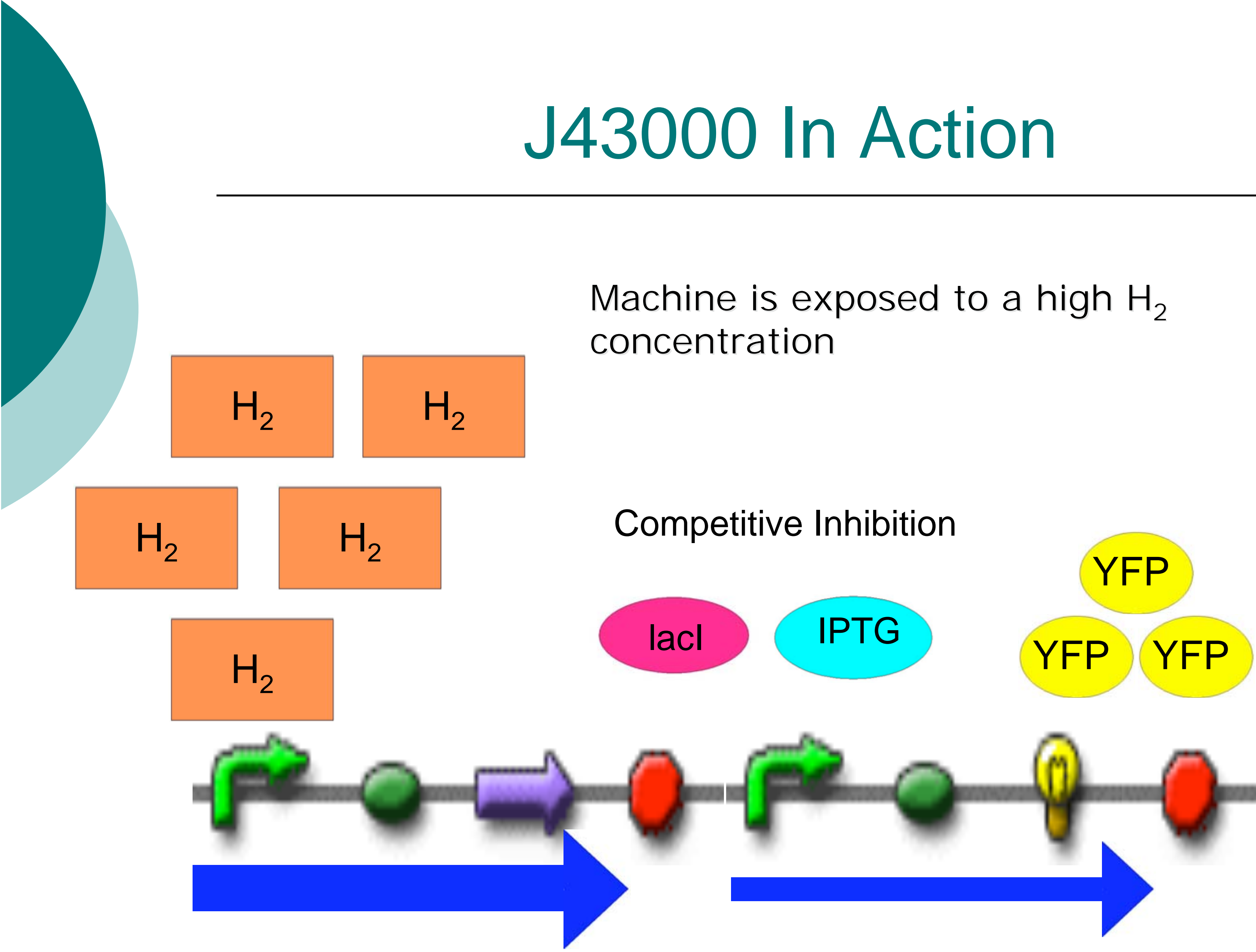
J43000 In Action

Lac promoter is further repressed, leading to lower YFP production



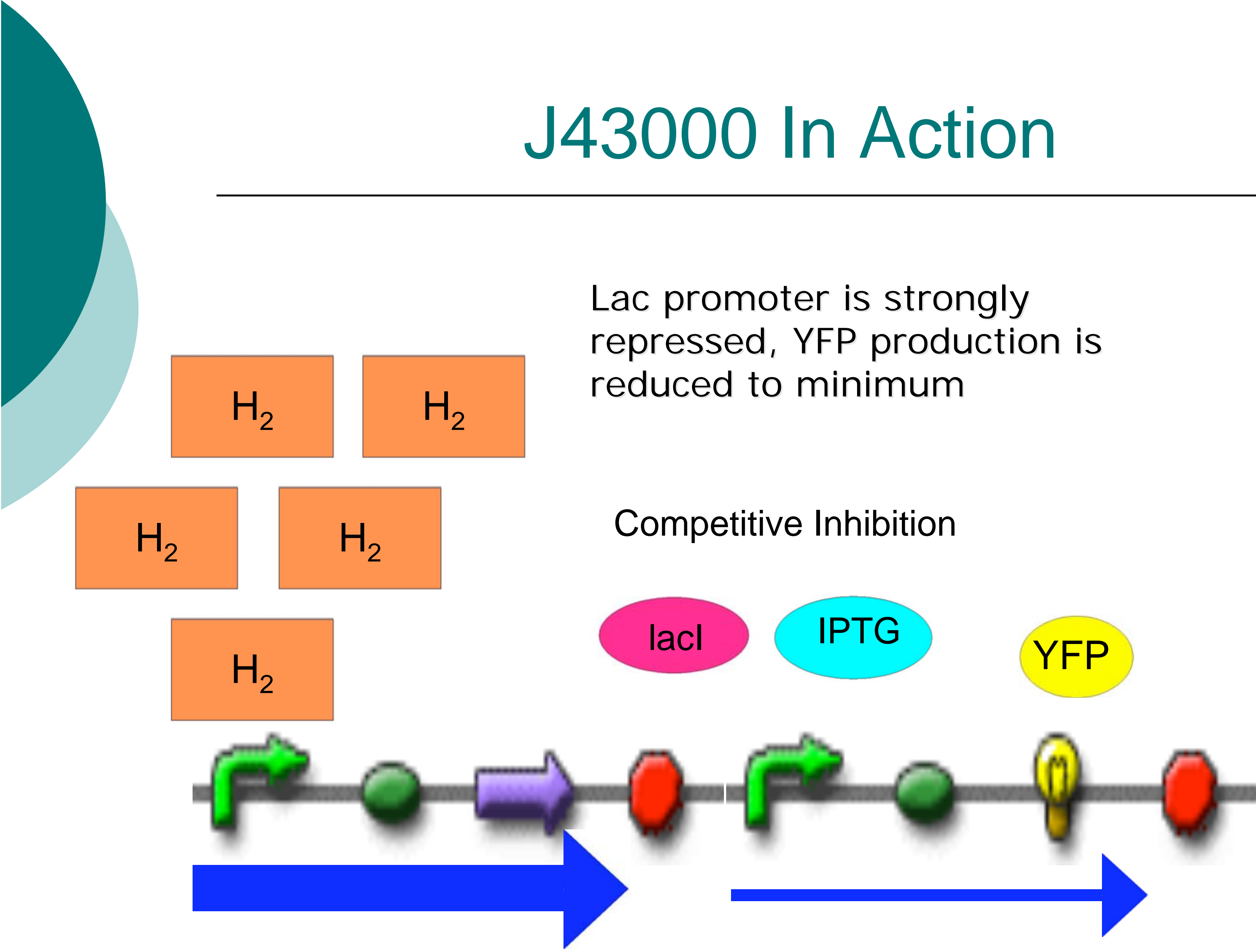
J43000 In Action

Machine is exposed to a high H_2 concentration



J43000 In Action

Lac promoter is strongly repressed, YFP production is reduced to minimum



Testing Our Machine

Requirements

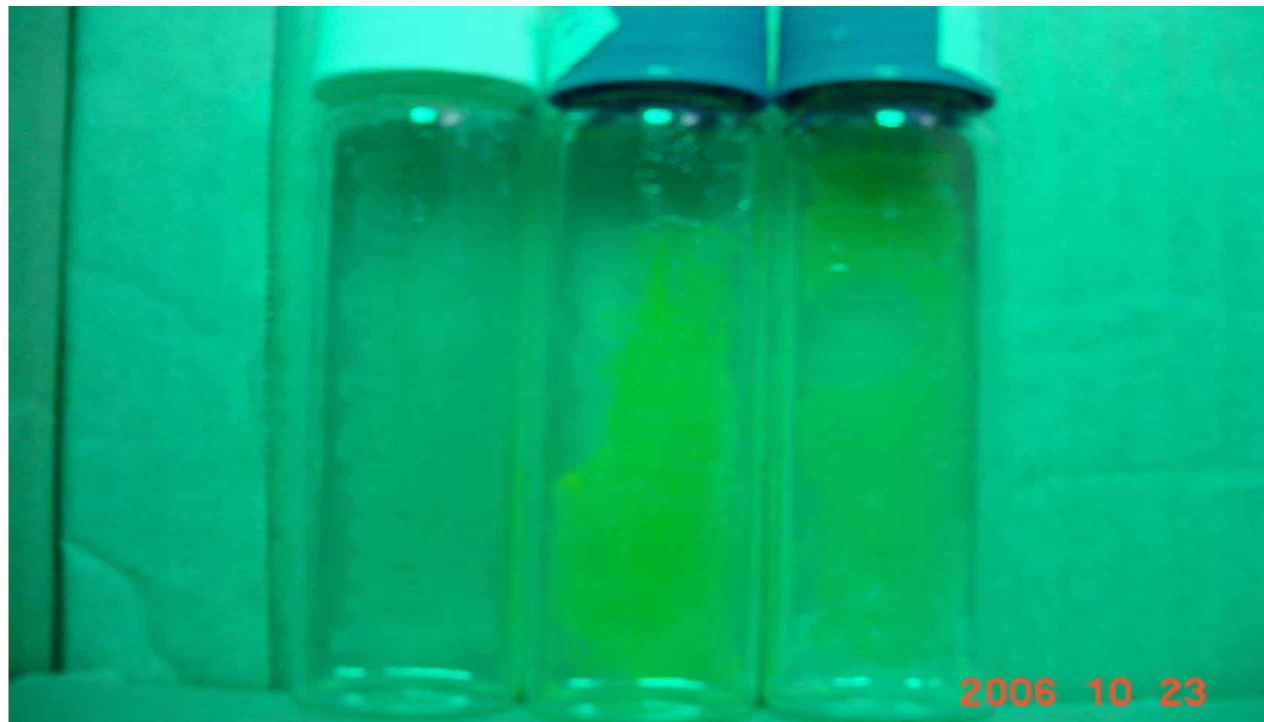
- Introduction of inducer (IPTG)
  - Provides a broader range in fluorescence levels
- Varied H_2 concentrations
- Fluorescence quantification

- Gasing
  - Cells were grown in septum topped vacuum ready tubes
  - A vacuum pump is used to extract air from the tube
  - Each tube is filled with a specific H_2 concentration

- Photos
  - The samples were exposed to light wavelengths close to YFP absorption wavelength
  - Pictures were taken using a digital camera with fixed settings
  - Pictures were taken before, immediately after, and 5 hours after gassing

Our Machine

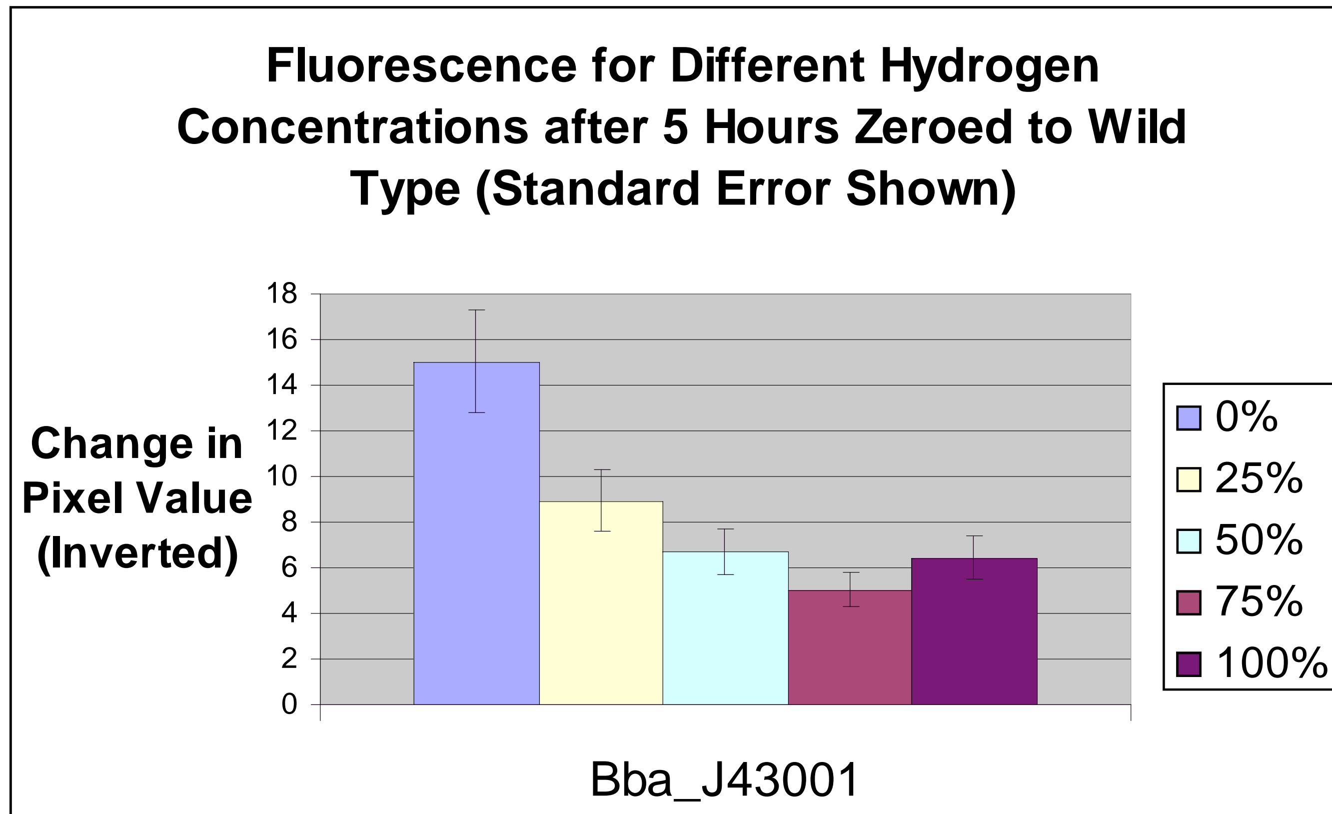
Visible fluorescence compared to unmodified E. coli



Testing Our Machine

- Analysis
  - MatLAB pixel analysis
    - Averaged pixel value for a selected area
  - 5 data sets were taken for each sample
  - Fluorescence change was calculated for pre-gassing samples and samples 5 hours after gassing
  - Changes were zeroed to wild type E.Coli fluorescence changes

Results



- Minimal YFP production without addition of IPTG
- Strong YFP production with addition of IPTG
- Proportional reduction in fluorescence with addition of H_2

Further Work Required

- Fluorescence in 100% H_2 was actually higher than fluorescence in 75% H_2
  - Why?
- Positive results were obtained after incubating for 5 hours
  - Detection is NOT immediate
- More precise testing using wavelengths specific to YFP absorption and emission

Acknowledgements

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  - Dr. Filip To, Dr. Din-Pow Ma, Dr. Todd French
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- MSU College of Agriculture and Life Sciences
- iGEM Ambassador James Brown
- iGEM Staff

- Controlled Lipid Synthesis
  - Produce efficient energy source from inefficient organic energy sources
- Water Splitting
  - Efficient, portable H_2 production
- Tar Digestion
  - Yield cleaner energy sources
- Insulin and/or Blood Sugar regulator
  - Diabetes Control