


# BioSketch:

A bacterial sketchpad driven  
by a UV- and heat-sensitive  
genetic toggle switch

## Harvard iGEM 2005 - BioSketch Team

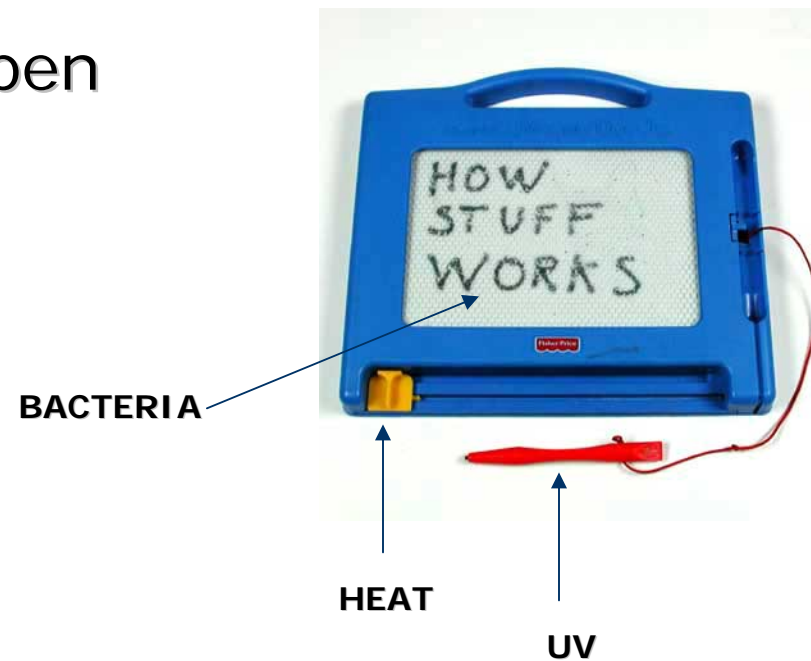
Christopher Doucette, Hing Eng,  
Jennifer Gao, Yin Li, Thomas  
Noriega, Yves Wang;

George Church, Radhika Nagpal,  
Kevin Parker, Ira Phillips, Pamela  
Silver, Alain Viel, Alexander Wait

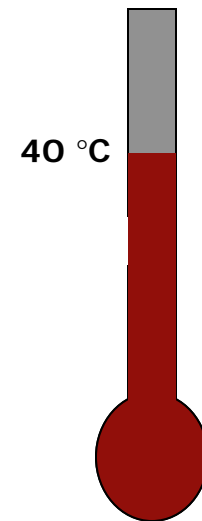
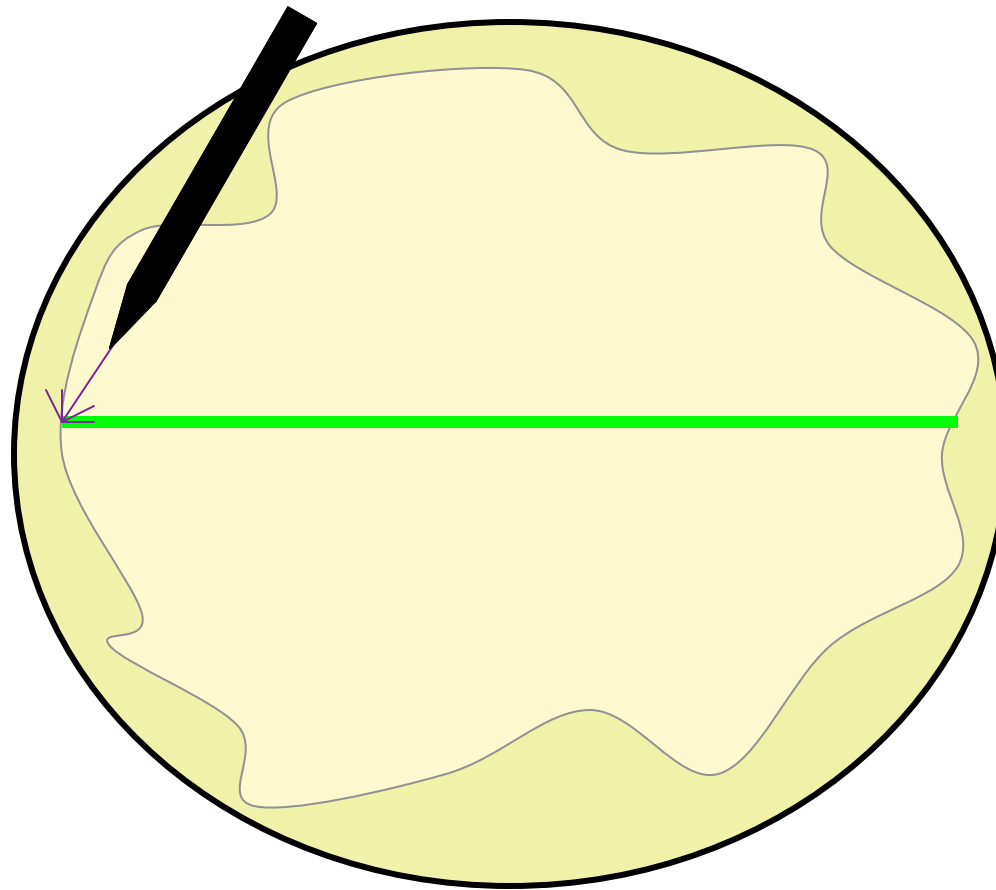


# Goal

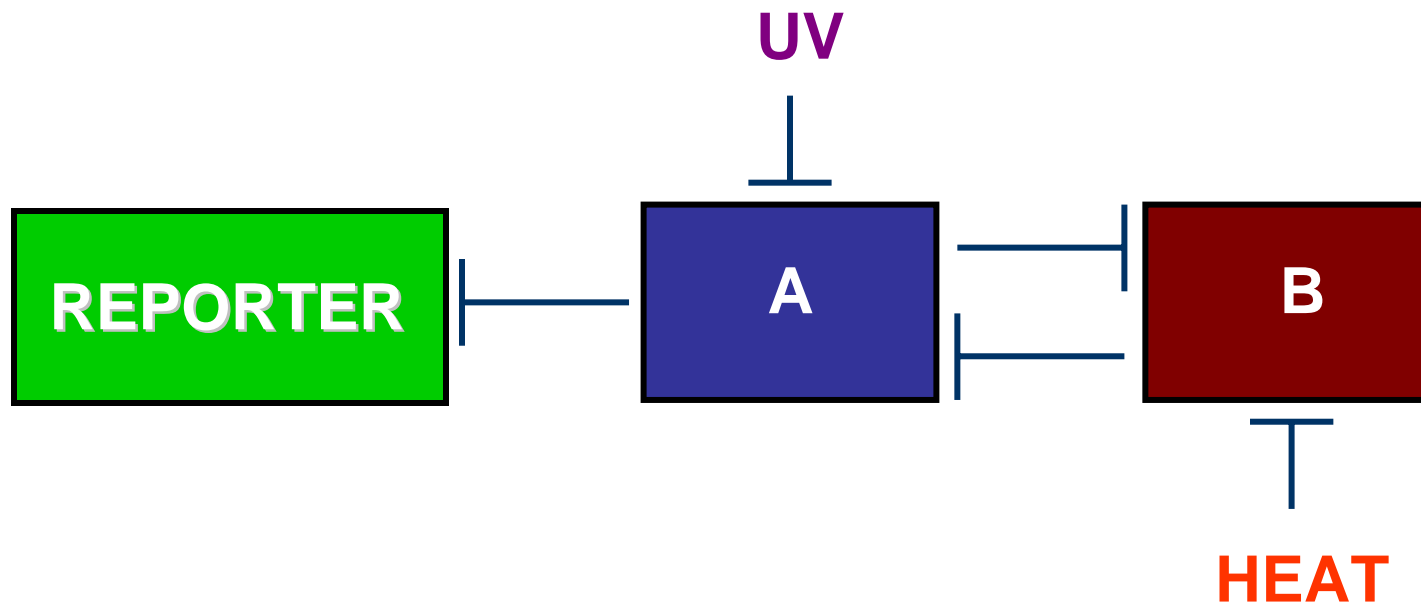
- Turn a lawn of *E. coli* into a reusable sketch pad
  - Write with a UV pen
  - Erase with heat



# Project Concept



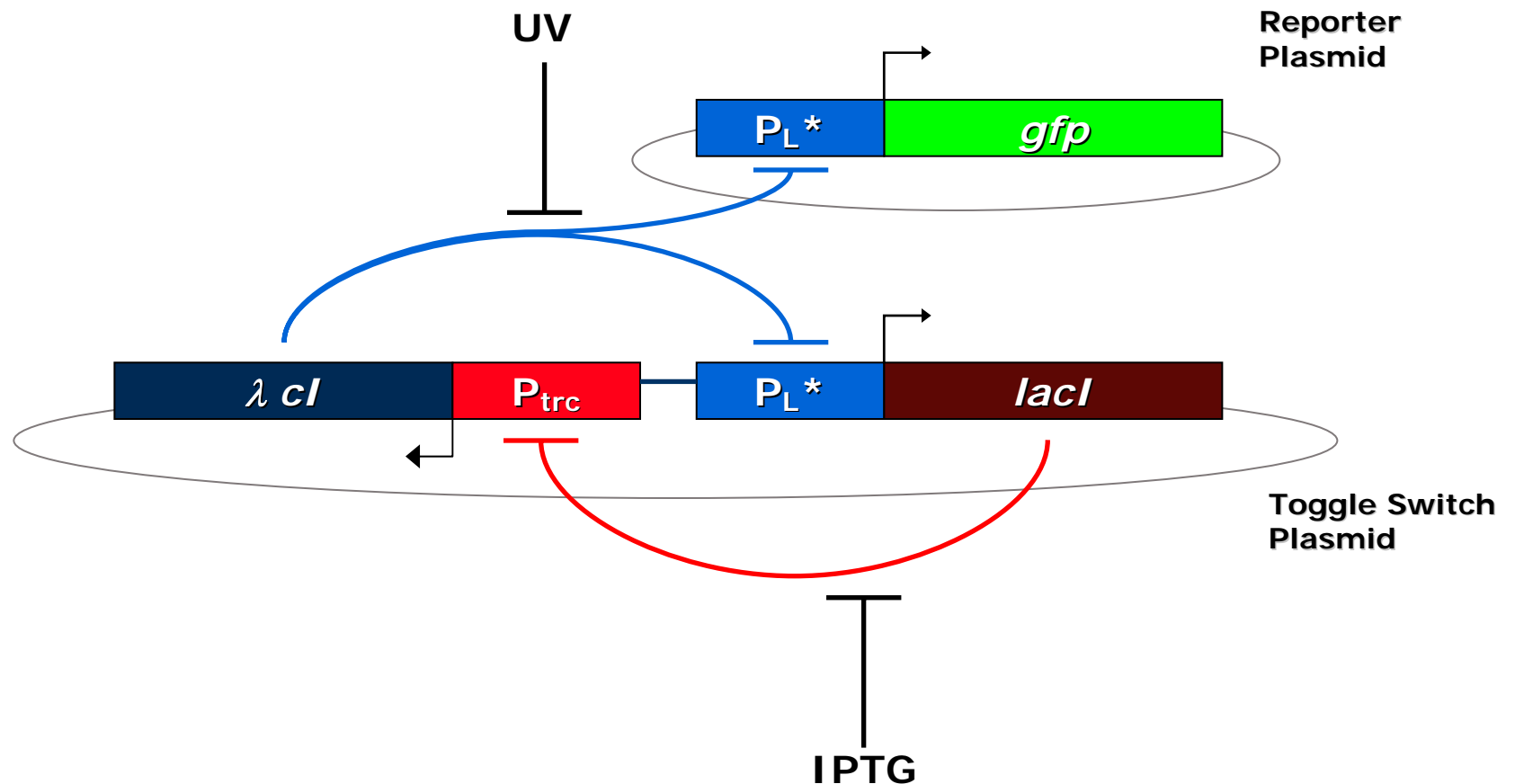
# Circuit Design: Toggle Switch



- A and B are transcriptional repressors
- Writing: A is cleaved in response to UV, allowing expression of B and reporter
- Erasing: Heat inactivates B, allowing expression of A

# The Toggle Switch Plasmid

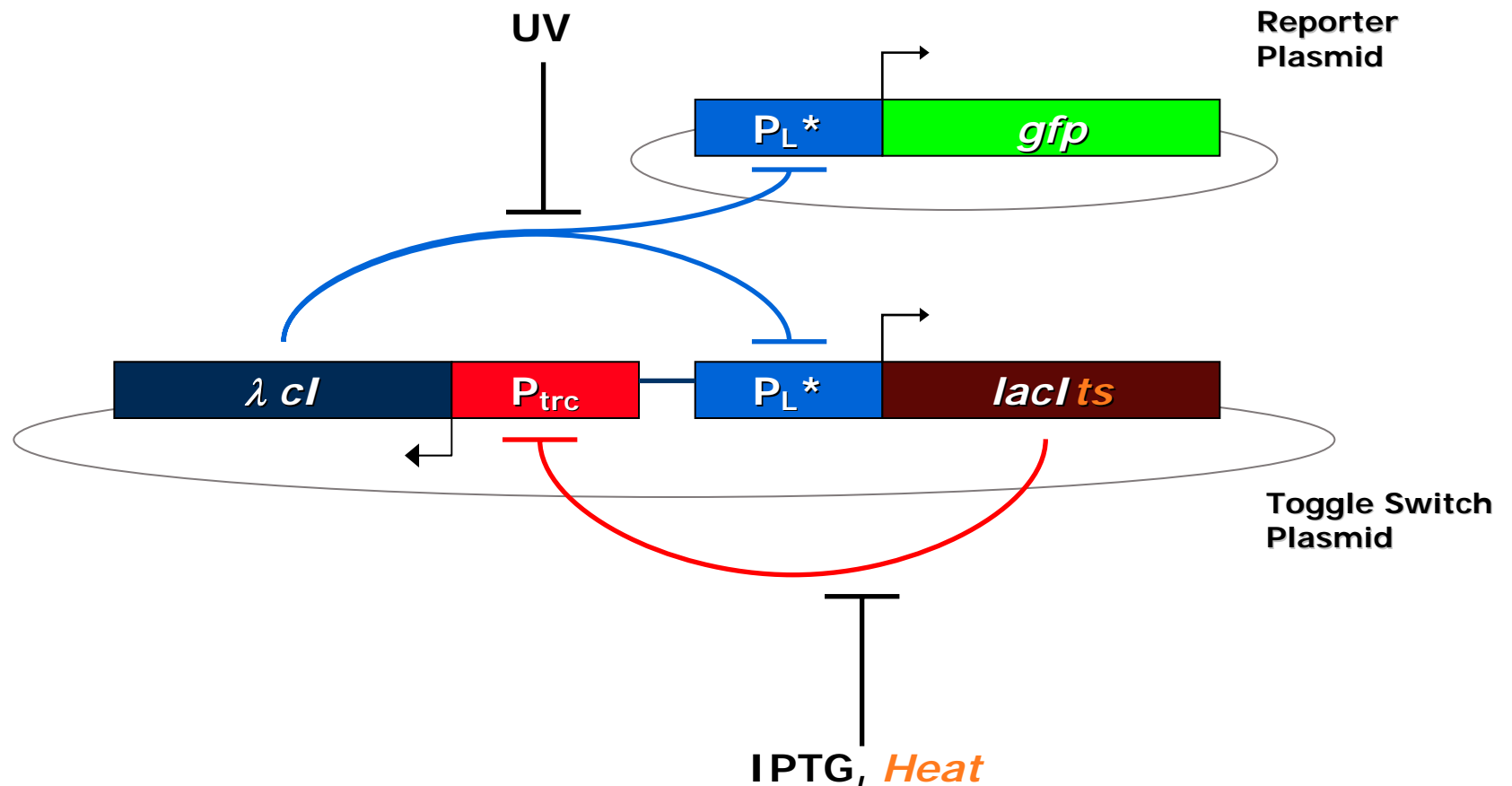
Original design from Collins lab:



Kobayashi, Kaern et al. "Programmable Cells: Interfacing natural and engineered gene networks." PNAS 101(22): 8414–8419 2004.

# The Toggle Switch Plasmid

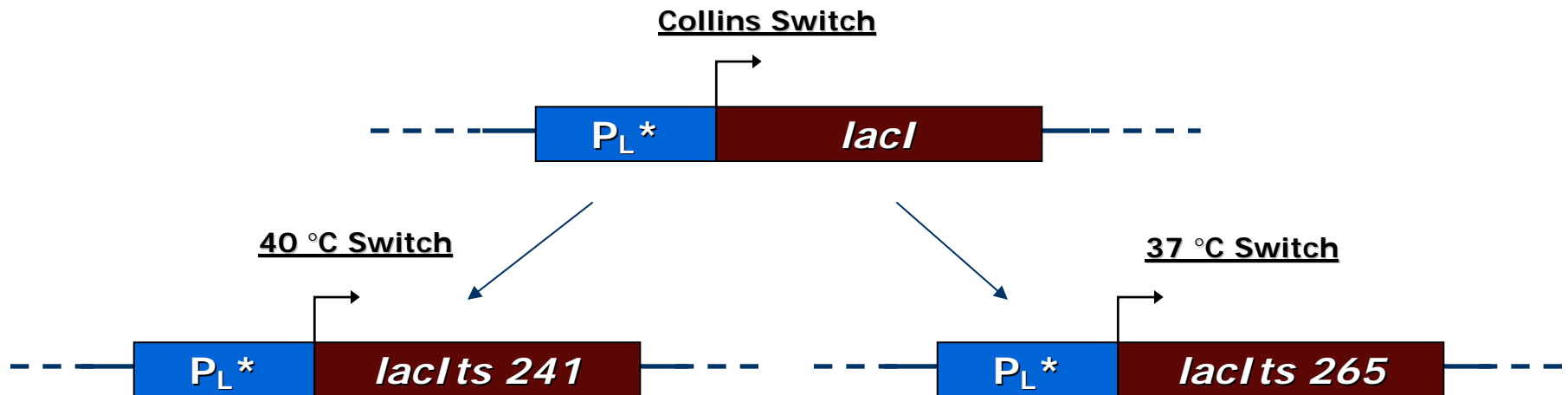
## Modified design for BioSketch:



Kobayashi, Kaern et al. "Programmable Cells: Interfacing natural and engineered gene networks." PNAS 101(22): 8414–8419 2004.

# Adding Heat Sensitivity

- Two temperature sensitive mutants of LacI repressor
  - Ala 241 → Thr: Repression lifted at 40 °C
  - Gly 265 → Asp: Repression lifted at 37 °C
- Site-directed mutagenesis of Collins plasmid



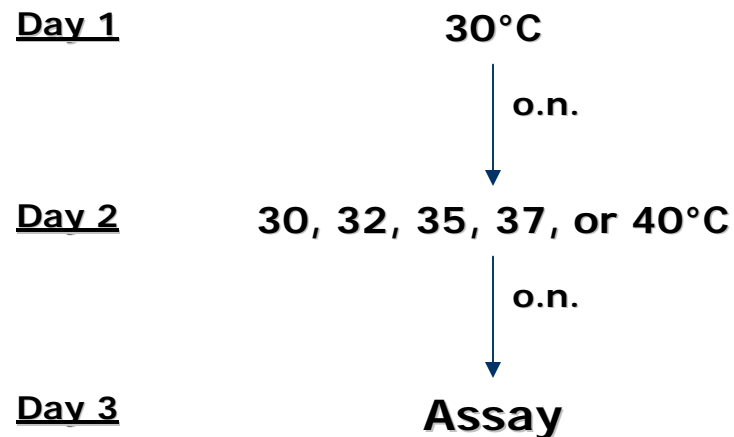
# Testing the Circuit

- Two  $\lambda$  cl / lacI<sub>ts</sub> switches built
- Can we set the OFF state with heat?
- Can we set the ON state with UV?
- Are states maintained over time?
- Assays:
  - Fluorescence microscopy
  - FACS analysis



# Testing: Turning Switch OFF

- Experimental Design

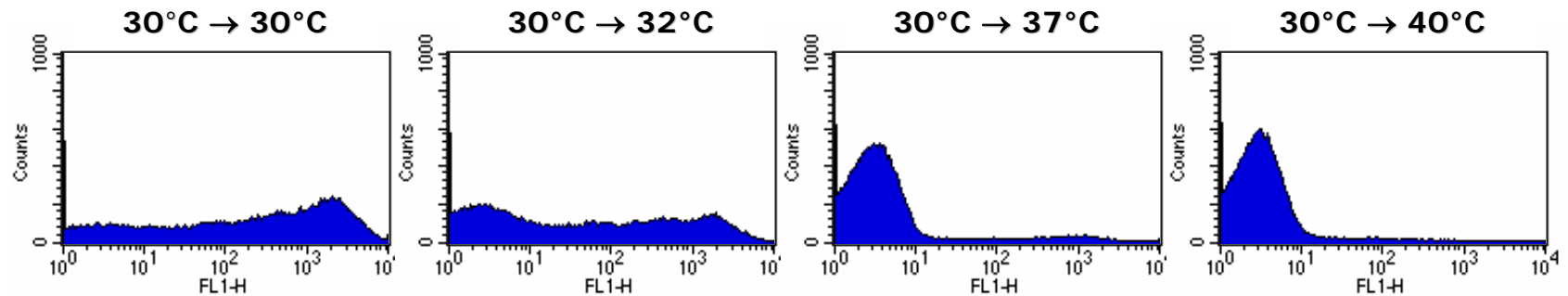


- Strains (LacI<sup>-</sup>)

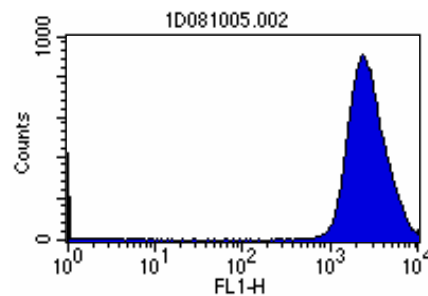
- 40 °C or 37 °C Switch
- ON state Control: GFP reporter only
- OFF state Control: No construct

# Testing: Turning Switch OFF

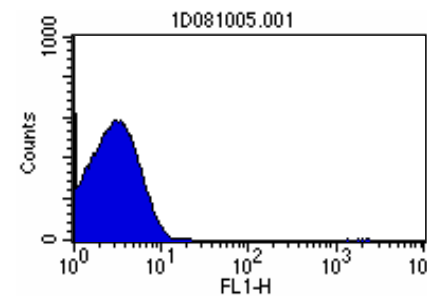
## 40 °C Switch



## GFP only



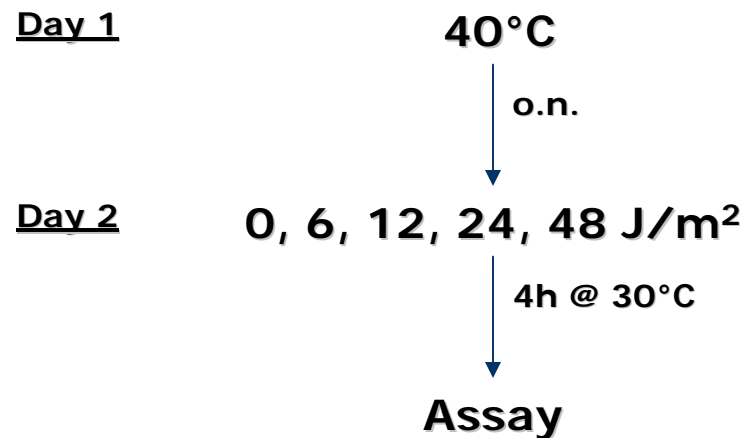
## No construct



- Incubation at 37-40°C almost completely abolishes fluorescence.
- Increasing temperature had no effect on fluorescence profiles of control strains.

# Testing: Turning Switch ON

- Experimental Design

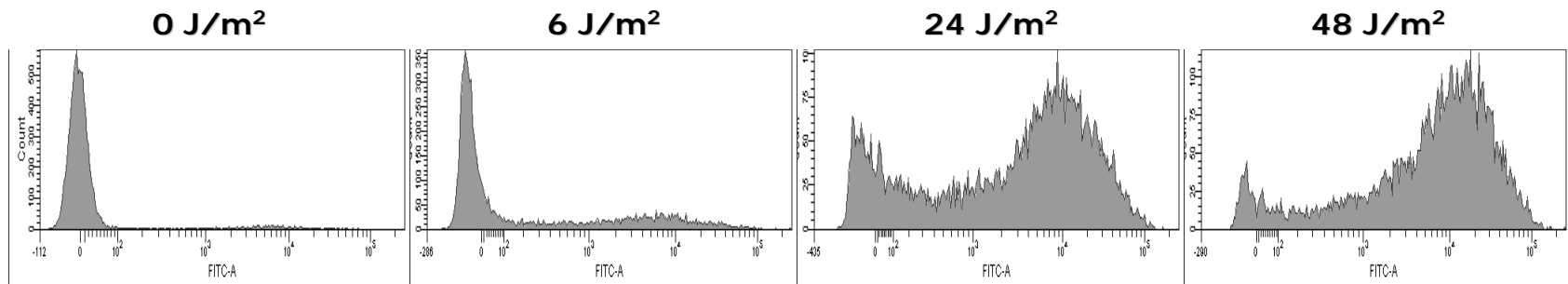


- Strains (LacI-)

- 40 °C or 37 °C Switch
- Positive Control: GFP reporter only
- Negative Control: No construct

# Testing: Turning Switch ON

## 40 °C Switch



- Increasing intensity of UV correlates with increasing intensity of cell fluorescence.
- UV irradiation had no effect on fluorescence profiles of control strains.

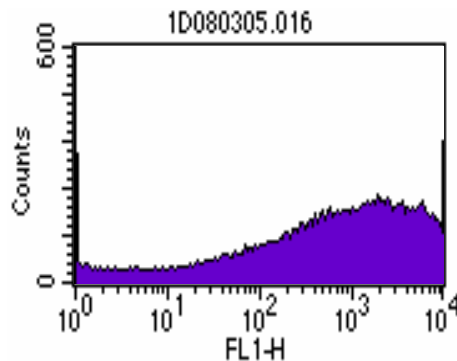
# Testing: State Maintenance

- 3 states for cell populations
  - ON (after UV exposure)
  - OFF (after heat exposure)
  - Mixed (some ON, some OFF)
- Populations in mixed state initially
- After cells are set to ON or OFF, state should be stable indefinitely

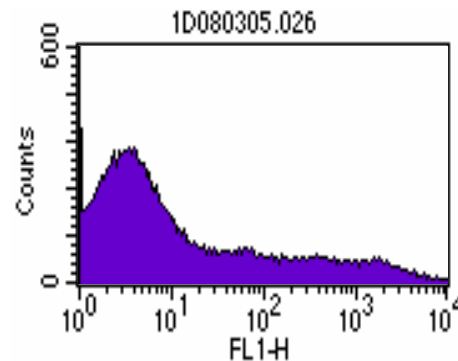
# Testing: State Maintenance

## 40 °C Switch

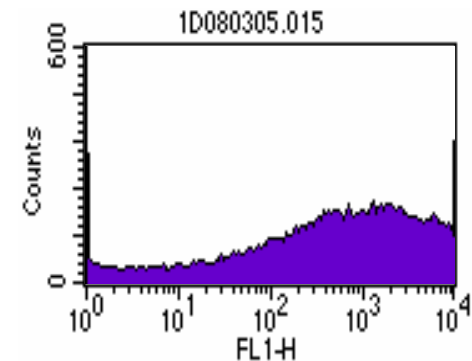
No heat treatment



2 hrs after heat



24 hrs after heat



- Population begins in mixed state.
- 2 hours after incubation, population is set to OFF state.
- 24 hours after incubation, population returns to mixed state.

# How To Improve State Maintenance

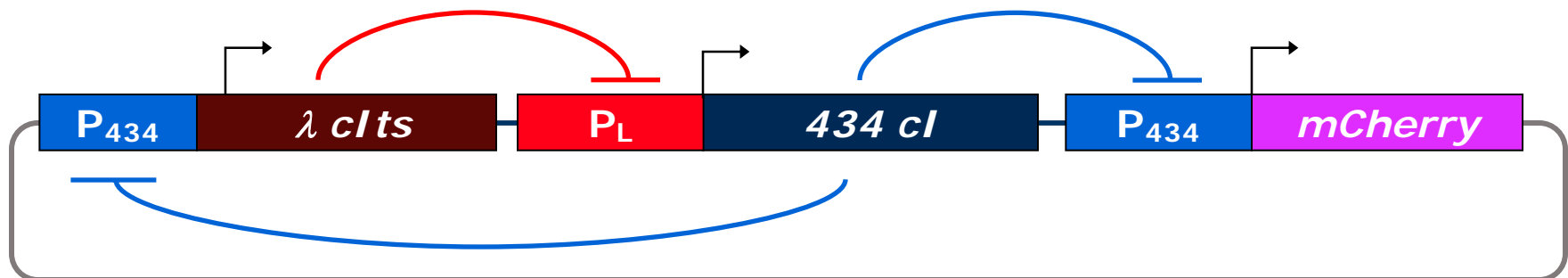
- For bistability, rates of repression and expression must be carefully balanced
  - Promoter strength & leakiness
  - Ribosome binding site strength
- Need to test multiple combinations of various promoters and RBSs
  - Send in the BioBricks!

# New Plasmid Designs

- Build from scratch using BioBricks from MIT Registry
- $\lambda$  *cl* / *lacI*ts switch:



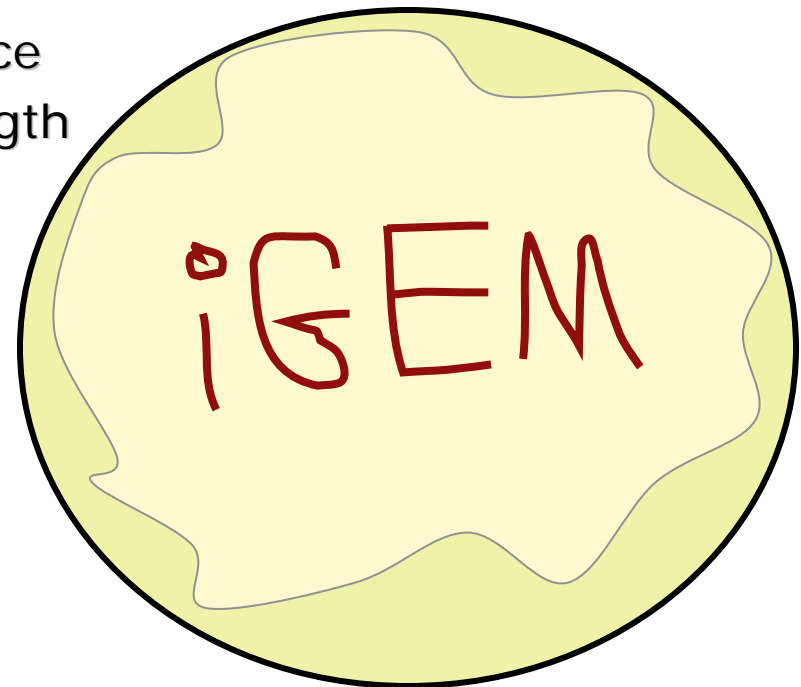
- 434 *cl* /  $\lambda$  *cl*ts switch:





# Summary

- Accomplished:
  - Turn switch ON with UV
  - Turn switch OFF with heat
- To Do List:
  - Get better induction with UV
  - More robust state-maintenance
    - Vary RBS, promoter strength
  - Test on solid media



# Acknowledgments

- Orr Ashenberg, Patrick Bradley, Connie Cheng, Kang-Xing Jin, Danny Popper, Sasha Rush
- Alain Viel, Ira Phillips, Sasha Wait
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- Jim Collins Lab (Boston University)
- Registry of Standard Biological Parts (MIT)
- Bauer Center Flow Cytometry Facility (Harvard)
- Dana Farber Cancer Institute Flow Cytometry Facility