ready, set, swarm!

designing a bacterial relay race

penn state iGEM 2006

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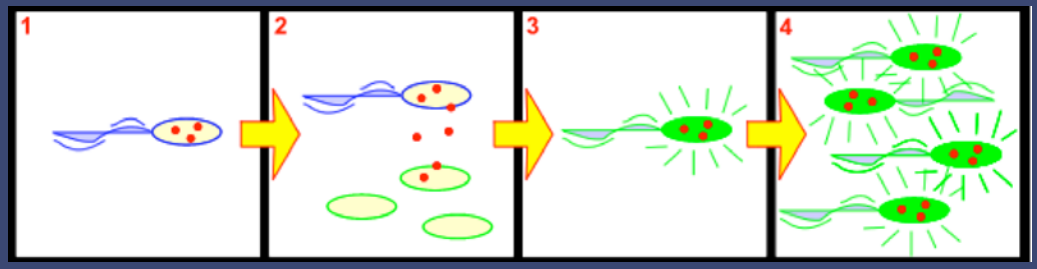
outline

- penn state team project idea
- system requirements/ approach to problem
- strategy
- subtasks
  - circuit design
  - micofabrication
- progress since iGEM ‘05
- future goals and challenges

concept

Idea: build a bacterial relay race

- motile bacteria move along a channel carrying a signal
- encounter a second immotile strain
- turn on a switch controlling the latter's motility

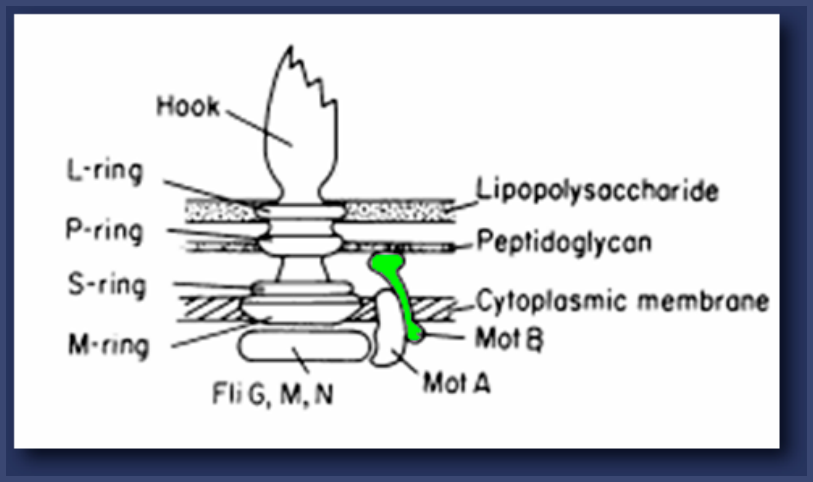


Why?

- Fun to bet on
- Great for lab downtime
- Novel signal carrier

system requirements

How to accomplish?



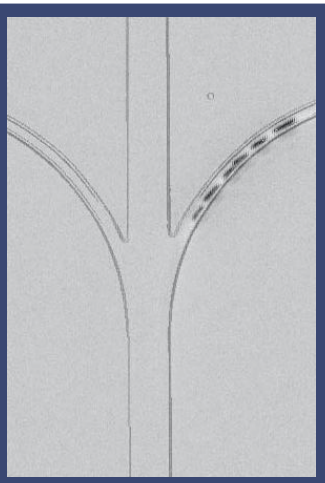
- **needs**
  - ***method to control movement***
  - way to direct movement

- **solution: control MotB flagellar protein**
  - Blair and Berg[1] showed that flagellar rotation could be restored in MotB K/O cells by complementing with a functional copy on a plasmid
  - rotation restored on average in 10 min

[1]Blair, D., Berg, H.G. Restoration of Torque in Defective Flagellar Motors. *Science* 242, 1678-1681 (1988).

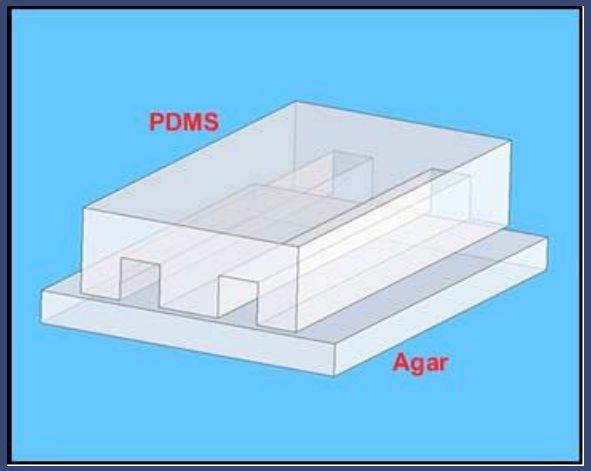
system requirements

How to accomplish?



- **needs**
  - method to control movement
  - ***way to direct movement***

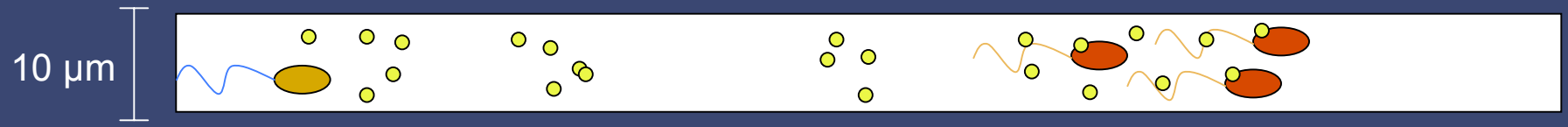
- **solution: microchannels**
  - offer facile method for guiding bacteria
  - no gradient necessary - Whitesides & Berg[2]
  - optimal environment to constrain and direct quorum signal



[2]Berg, Whitesides, et al. E. Coli swim on the right. *Nature*, 435, June 30, 2005.

strategy

sender cells continuously produce AHL



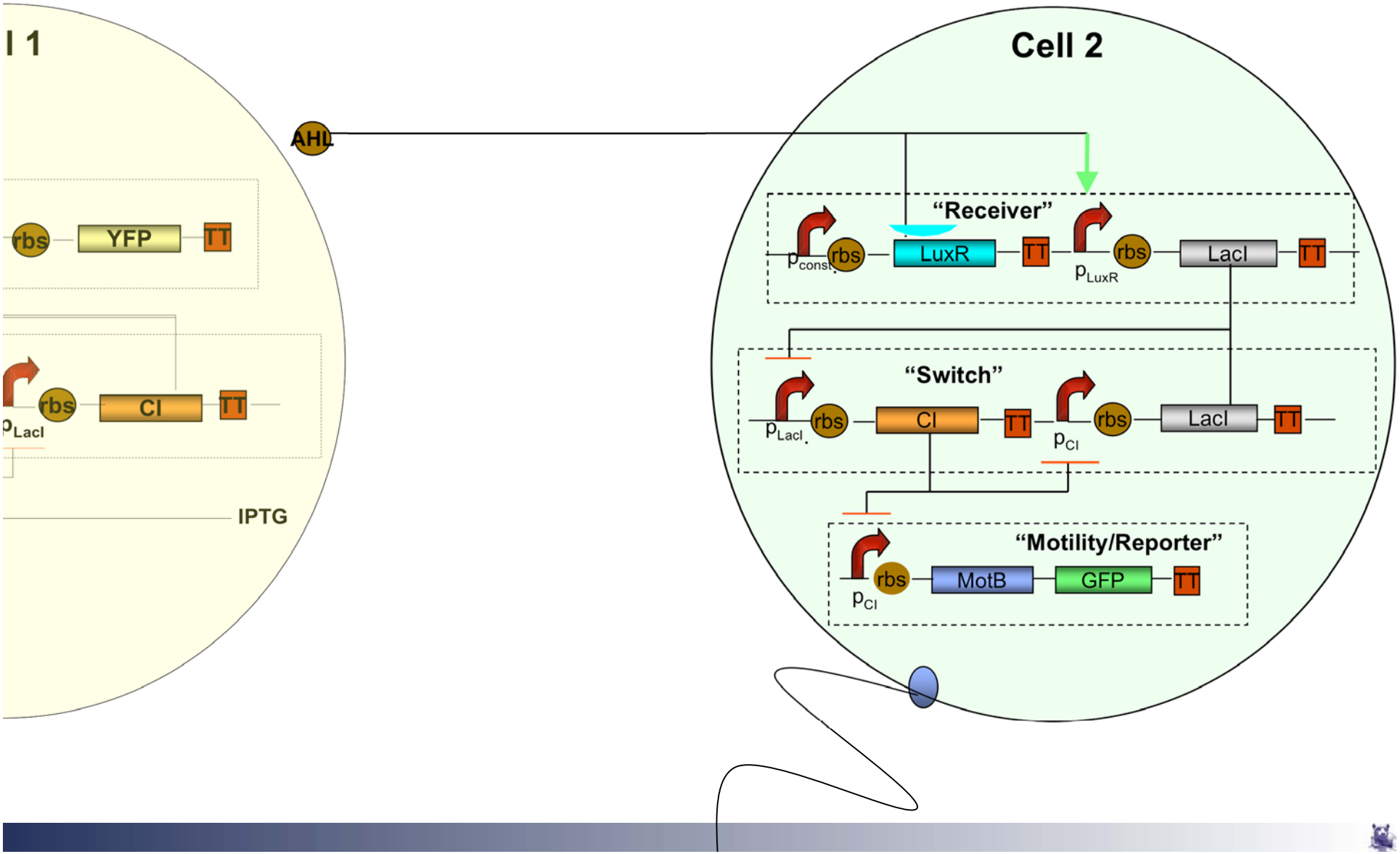
advantages

diffusible quorum signals have been functional activators in previous synthetic networks with luxR/AHL-controlled promoter

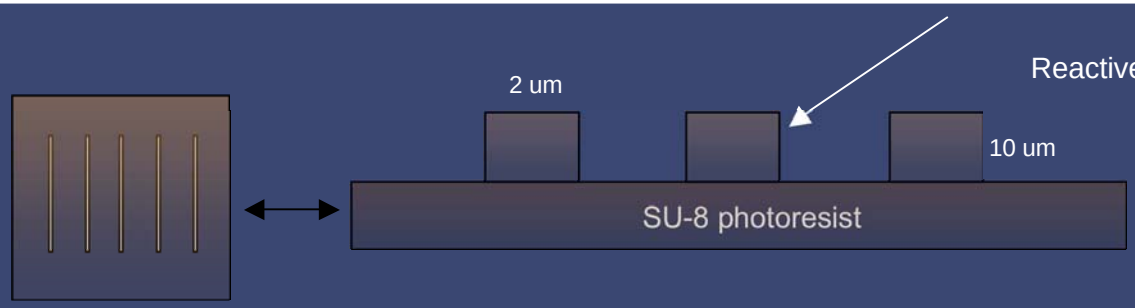
potential drawbacks

inadequate production of AHL for activation?; leaky expression from p_{luxR}

genetic control mechanism



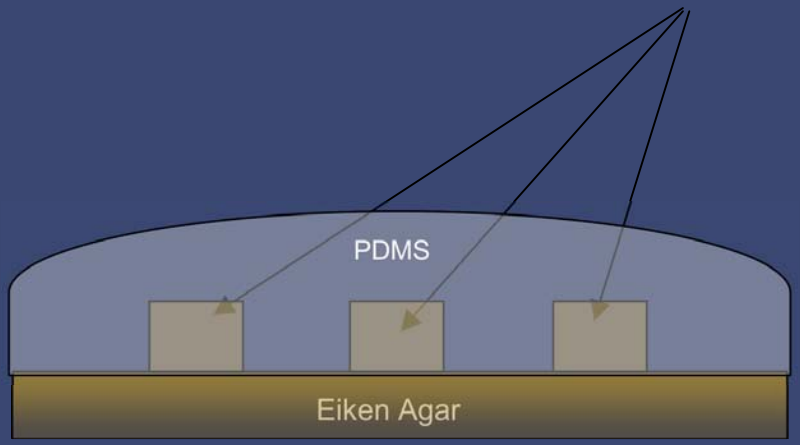
microchannel fabrication



Reactive-ion etched/photolithography w/mask

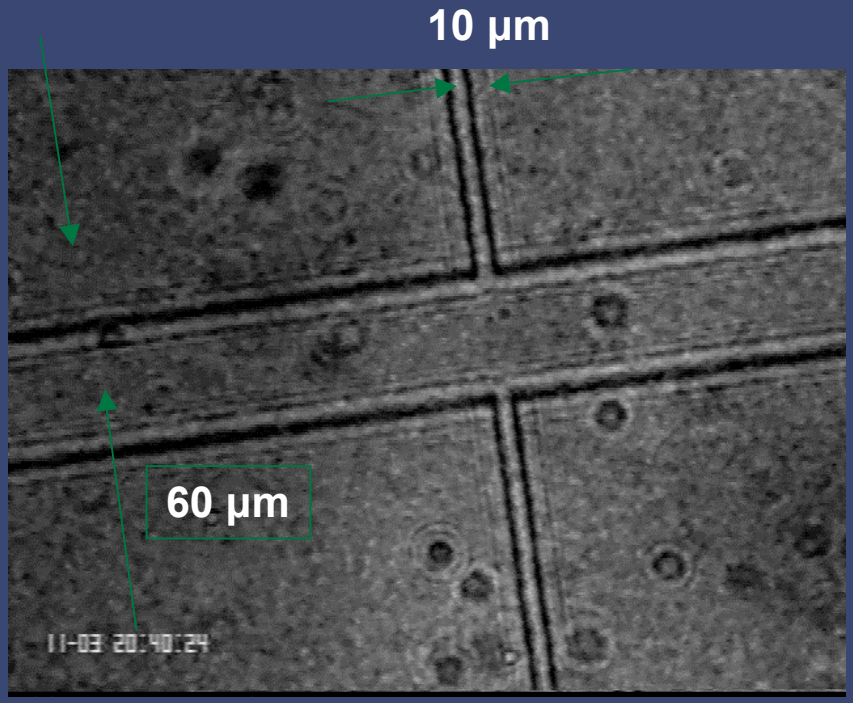
Pour PDMS, spin coat
At 200 rpm for 20 sec,
cure 4 hrs
@ 70 °C

Fluid-filled microchannels



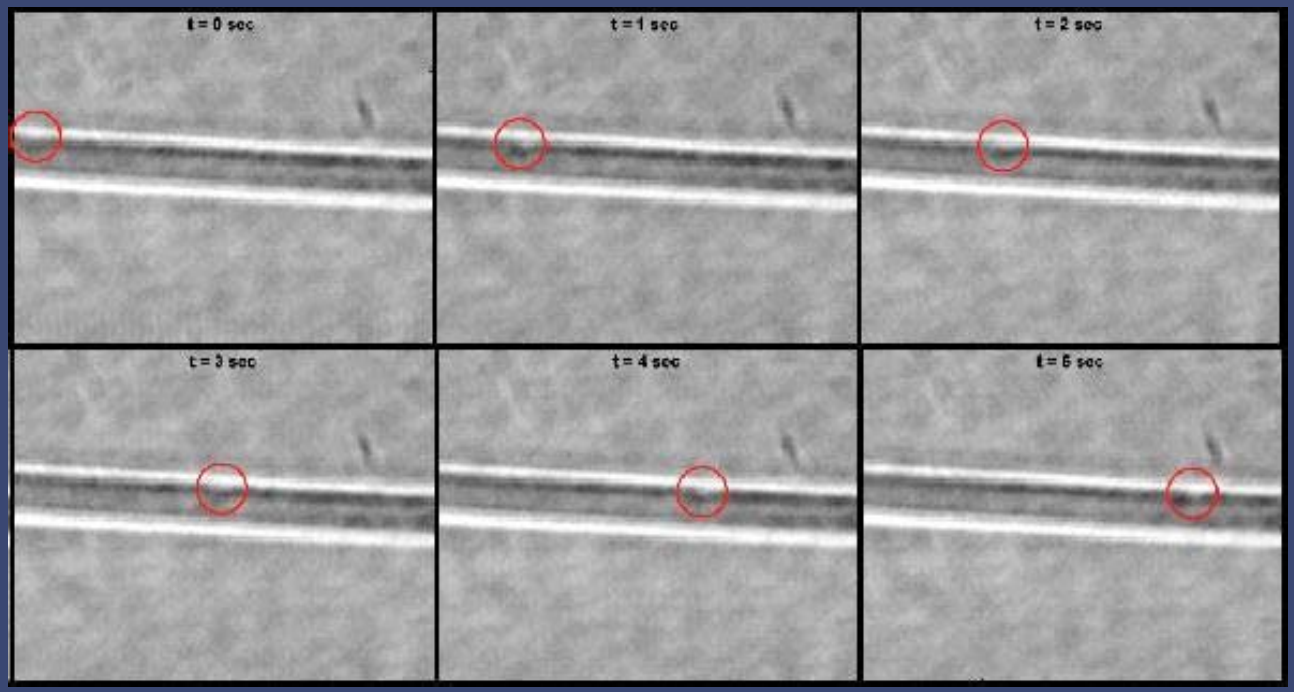
Peel off PDMS, place feature-side down
on agar (eiken) plate

microchannel pictures

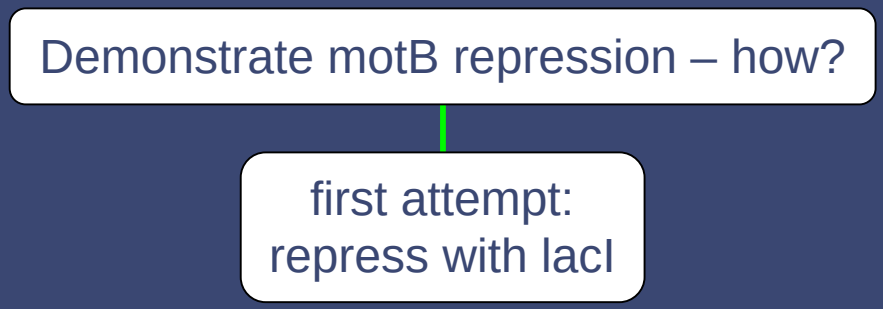


microchannel pictures

- cells swarming through our microchannels
  - **velocity of swarming:** ~10 μm s^{-1}

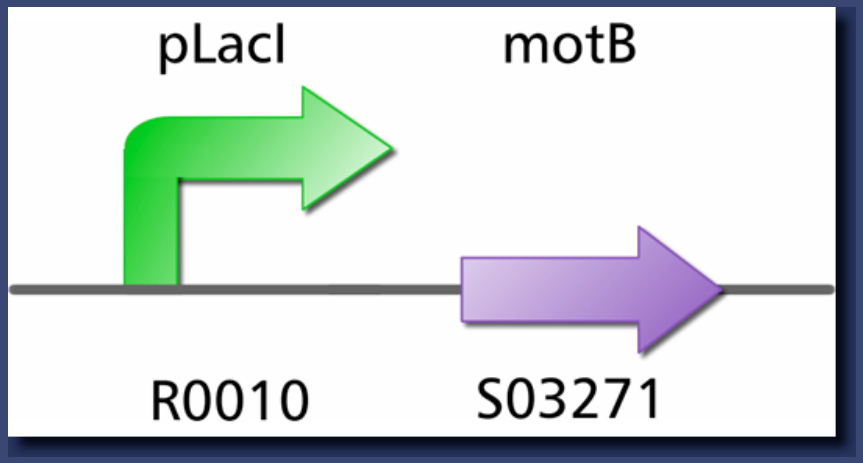


progress since iGEM 2005

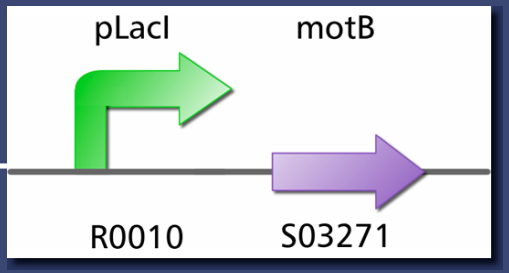


repression with lacI

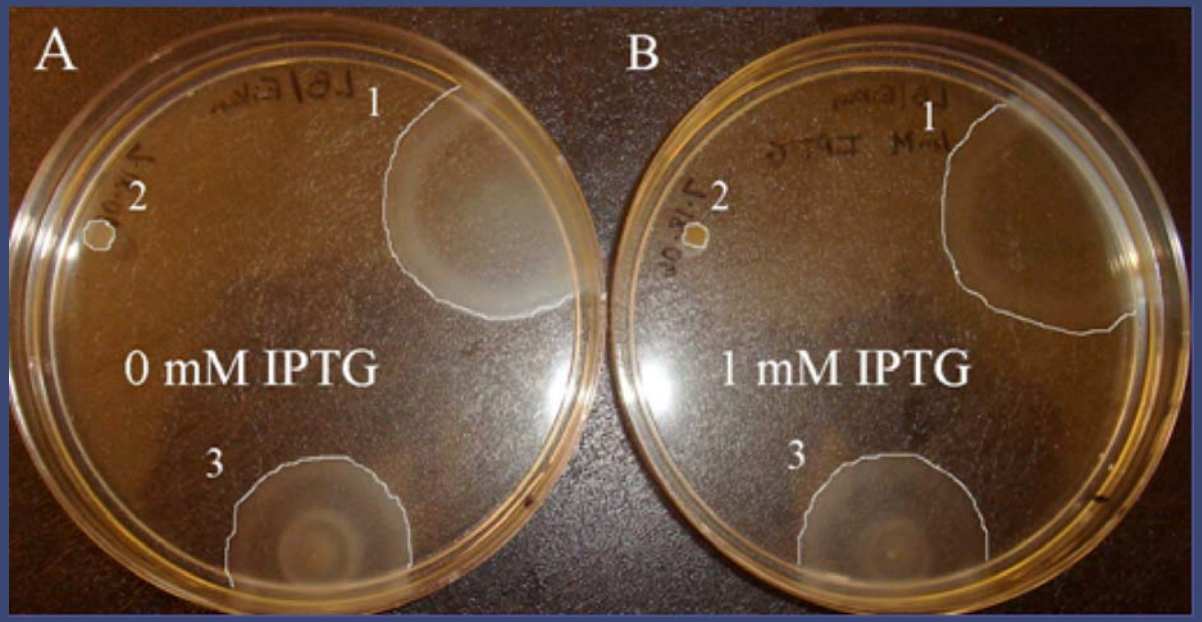
- crucial element of the project:
  - show repression of motB and induction upon desired input
- simplest construct to test repression and induction
  - place motB under control of lacI promoter



lacI repression results



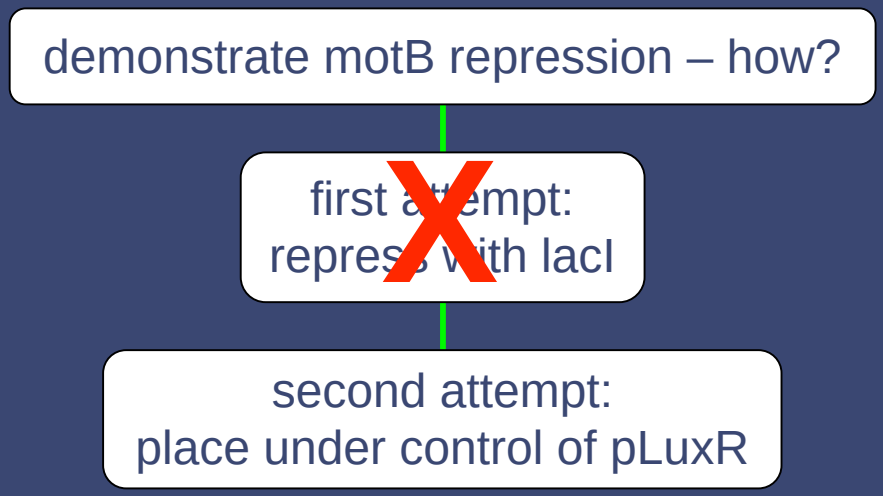
- **designations:**
  - **1** +control, strain RP437[3] (wild-type for motility)
  - **2** -control, strain RP3087[3] (motB$^-$)
  - **3** RP3087 with above construct at low copy (pSB4A3)



[3] Block, S. M. & Berg, H. C. Successive incorporation of force-generating units in the bacterial rotary motor. (1984) *Nature* 309, 470–472.

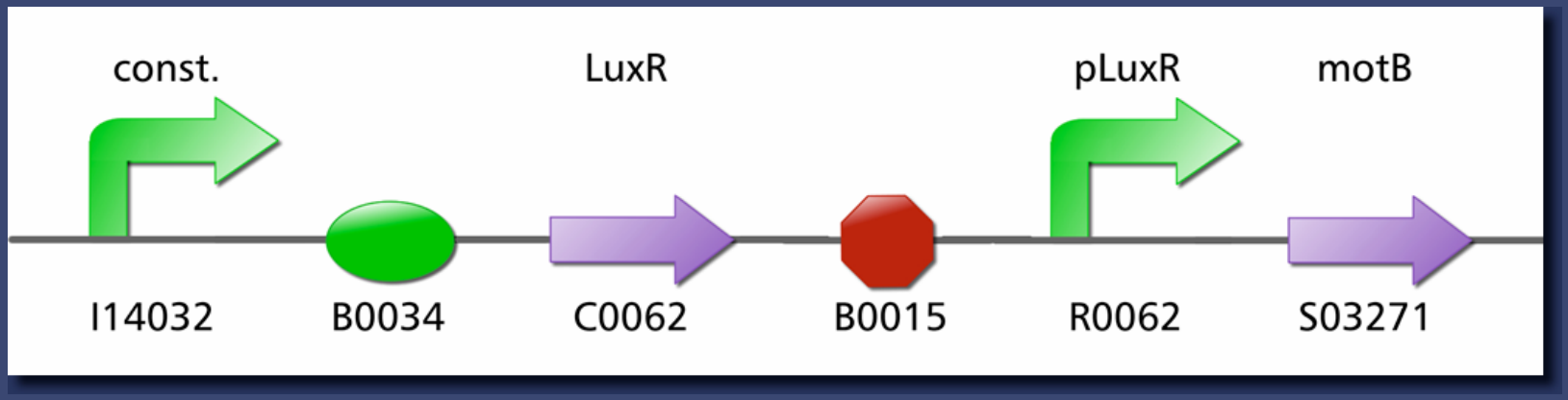
motB repression strategies

motB repression strategies

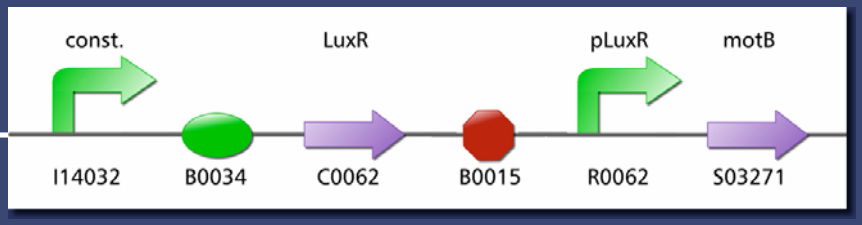


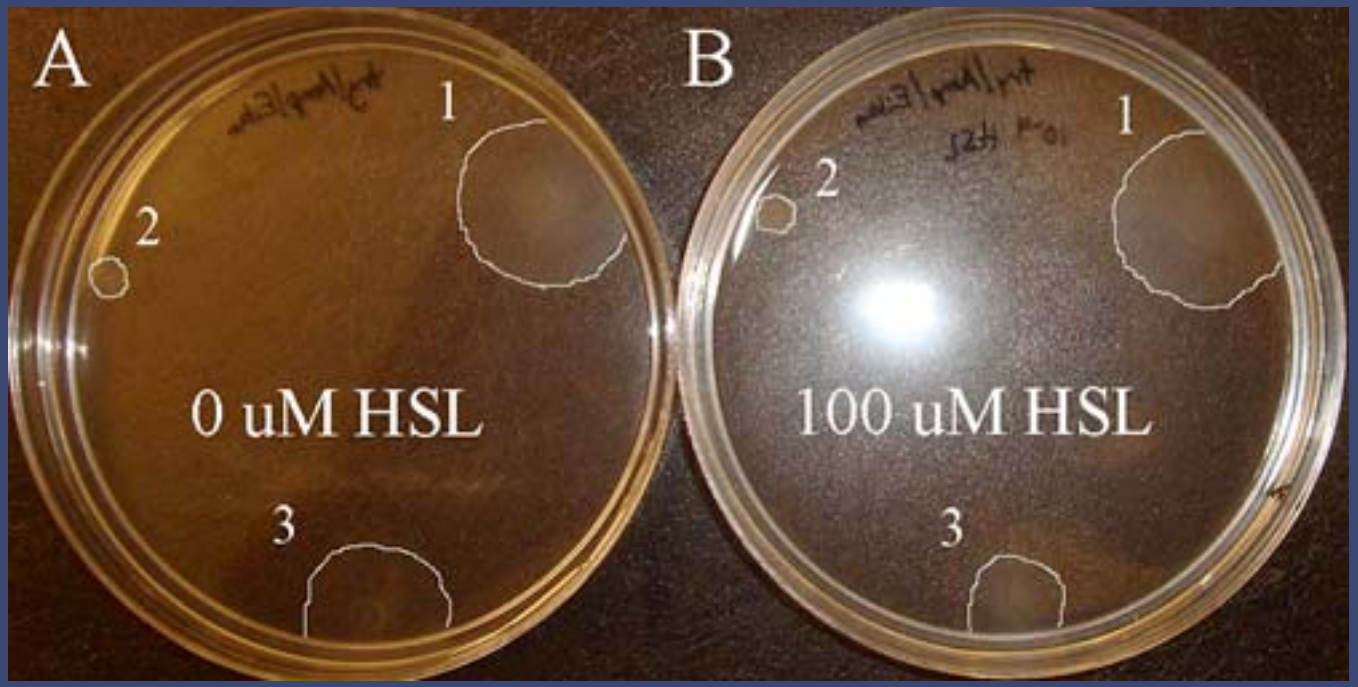
induction with HSL

- test induction of motB with HSL
  - use endogenous RBS of motB (BBa_S03271)
    - thought to be strong
- goal: examine leaky expression from pLuxR



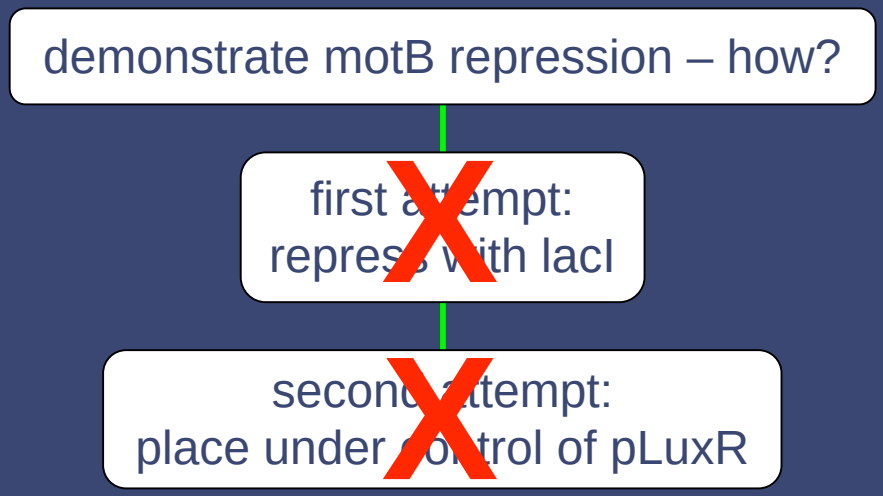
induction with HSL



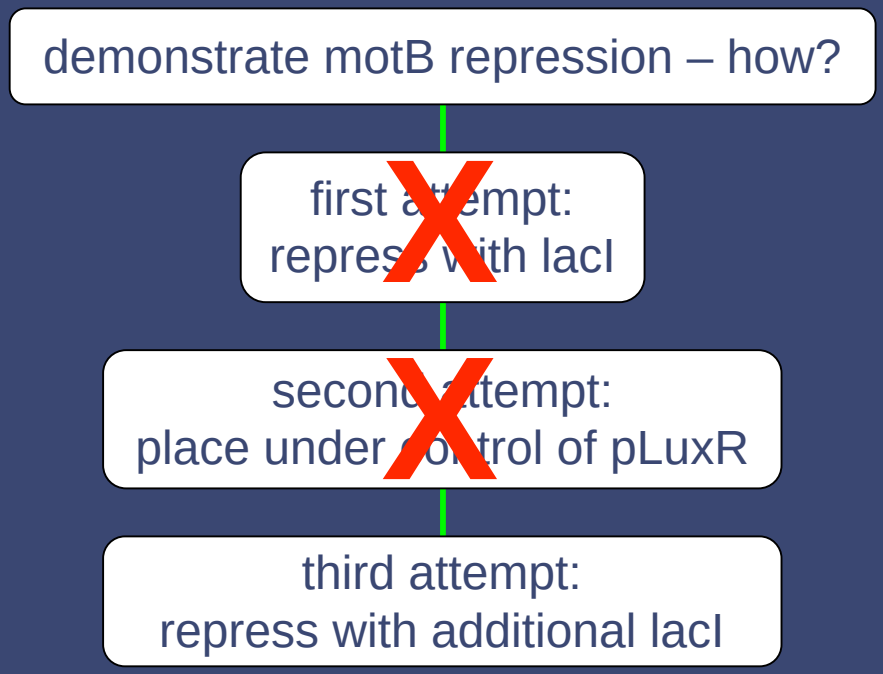


- **designations:**
  - **1** +control, strain RP437 (wild-type for motility)
  - **2** -control, strain RP3087 ($motB^-$)
  - **3** RP3087 with motB under control of pLuxR at low copy

motB repression strategies

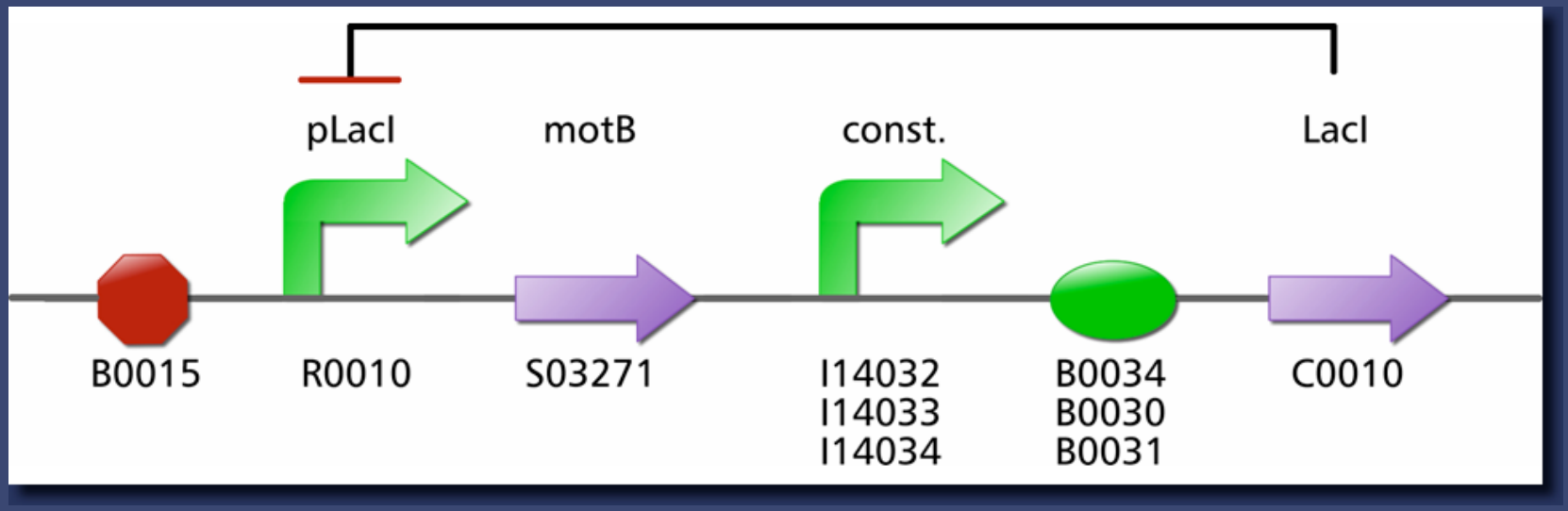


motB repression strategies

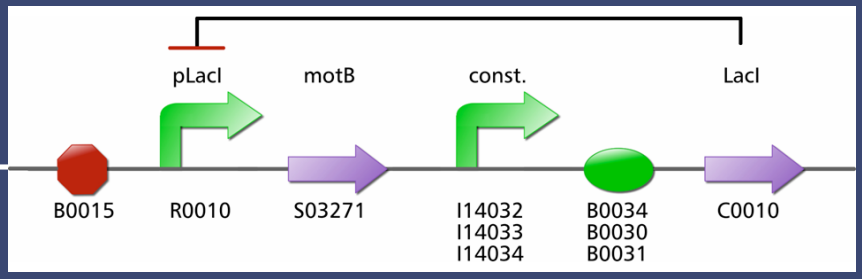


repression with additional lacI

- design another system to repress motB
  - necessary to show repression for project to work
- solution: place motB under control of pLacI
  - couple with additional expression of lacI
  - combinatorial approach
    - test library of promoter and RBS strengths



lacI repression, version 2.0



- **success!**
  - additional high consitutive expression of lacI shown to fully repress motB expression
- **designations:**

  1 +control, strain RP437 (wild-type for motility)

  2 -control, strain RP3087 (motB^{-})

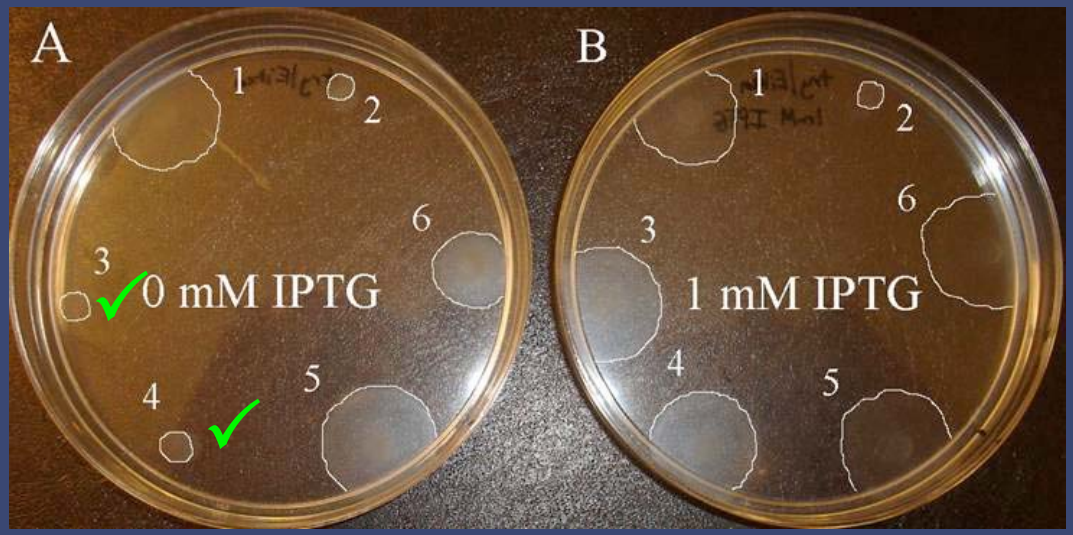
  3-6 contain construct above *at low copy (pSB4A3)*

  3 RP3087; I14032+B0034 (highest lacI output)

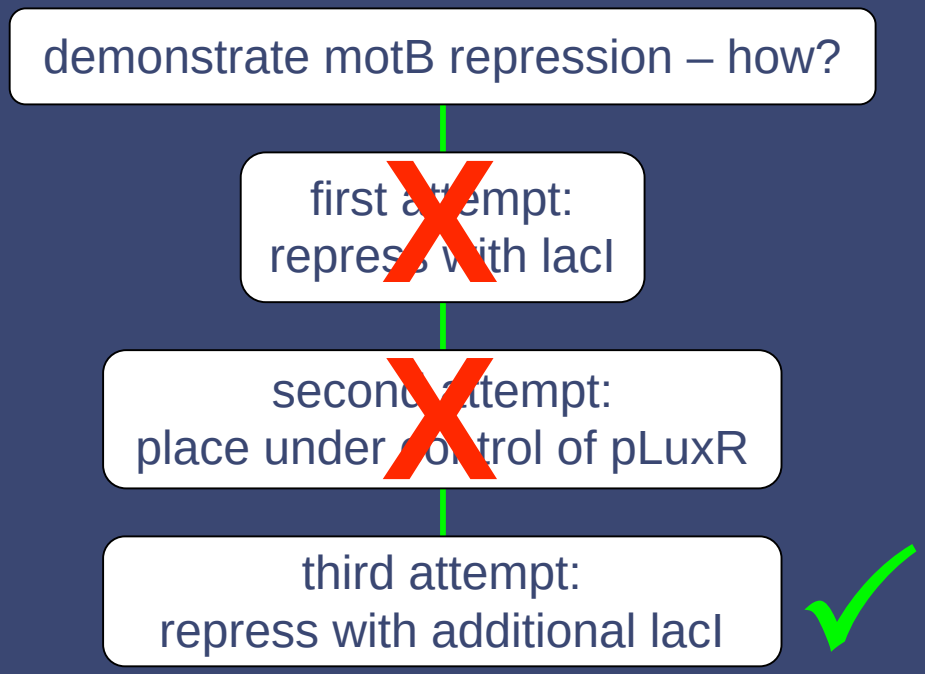
  4 RP3087; I14032+B0030 (high lacI output)

  5 RP3087; I14032+B0031 (medium lacI output)

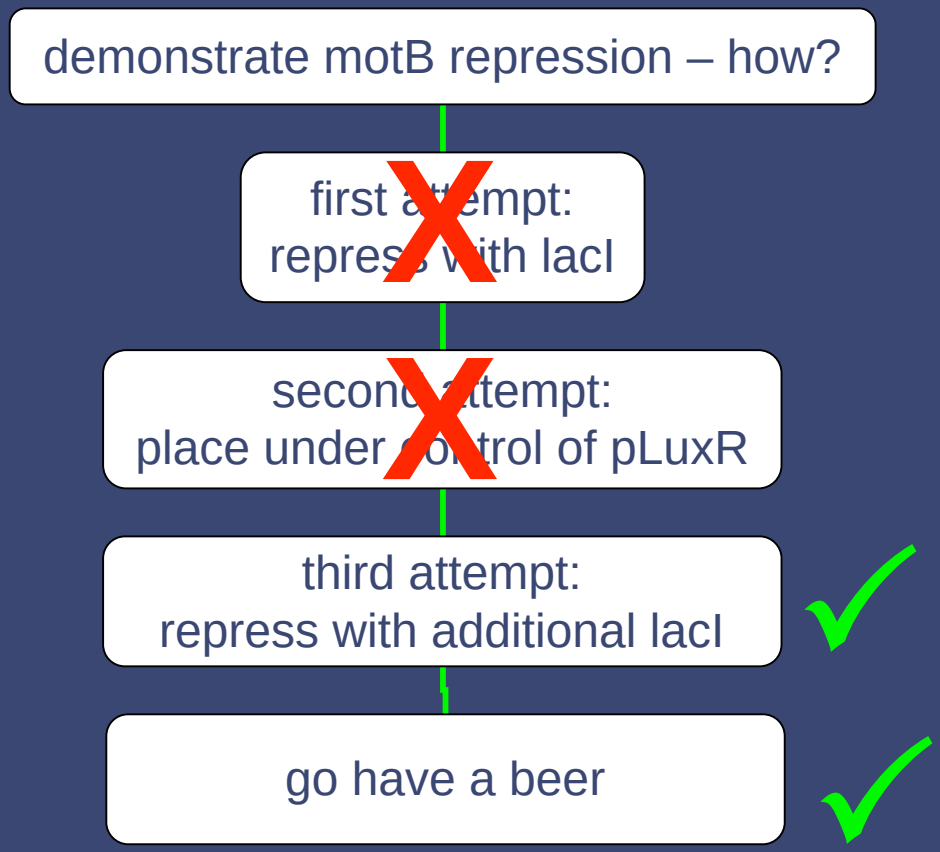
  6 RP3087; I14033+B0034 (medium lacI output)



motB repression strategies

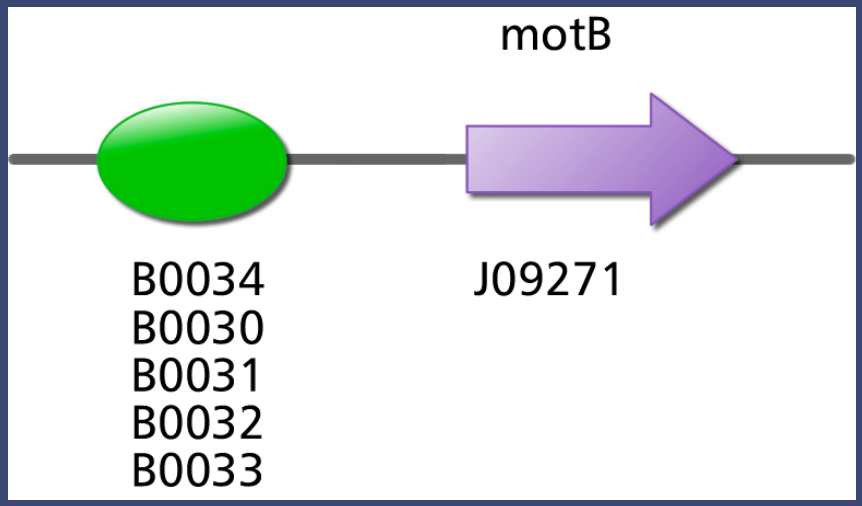


motB repression strategies



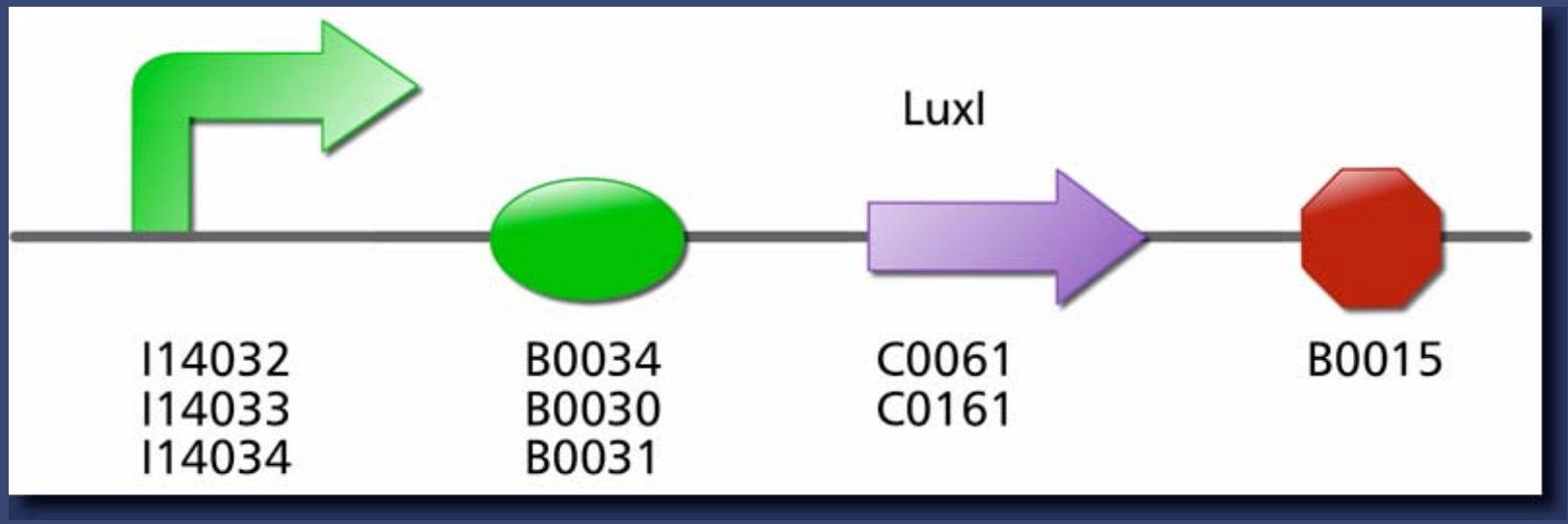
next steps

- results show repression of motB must be tight
- how to incorporate tighter repression with failed luxR input device?
  - remove endogenous RBS by PCR, add biobrick ends
  - make constructs with range of RBS strengths
    - in progress
- new part: **BBa_J09271**, motB without RBS



sender devices

- construction of sender cell output devices
- combinatorial approach
  - allows for selection of best HSL producer to induce motility in the recipient



challenges

- combinatorial approach to cloning
  - inability to “forward engineer” due to lack of characterization of part interactions
- demonstrate repression of motB under control of pLuxR
  - unknown to what degree RBS strength must be reduced
- determine level of HSL output necessary to induce motility in recipient
- visualization of quorum sensing events in microchannels

future work

- clone library of motB with varying RBS strengths
- test for repression, induction with HSL
- examine possiblity of antisense RNA to tighten pLuxR leakiness
- induce recipient cels with HSL produced from sender cells
- visualize induction in microchannel via fluorescent reporters
- construct strains – knockout lacI in RP3087 ($motB^-$)
- test receiver cell with switch
- implement stopping mechanism in sender?

acknowledgments

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questions? answers?

Thank you!