Toward a Bacterial Internet: Addressable Bacterial Communication

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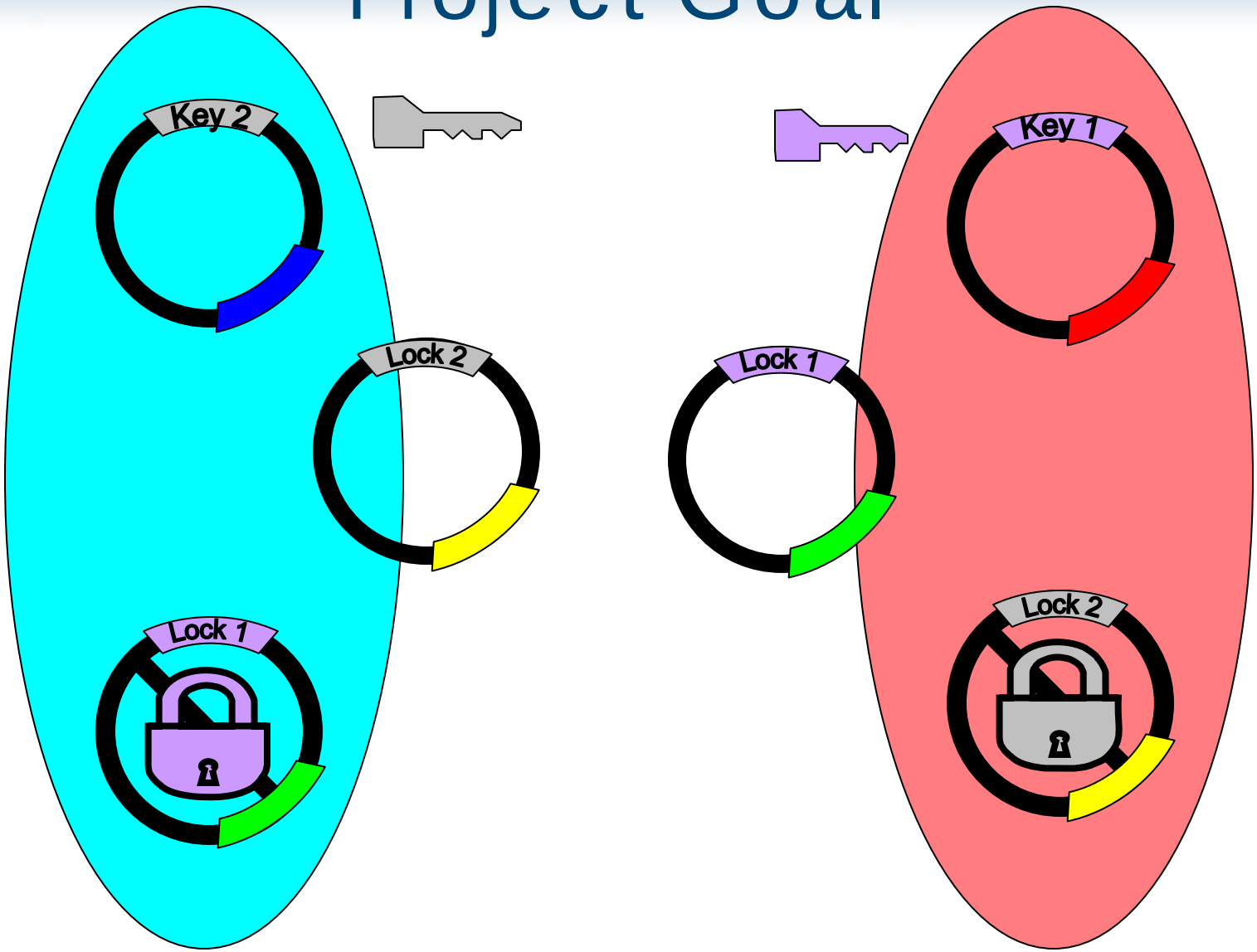
Overview

I. Project Goal
II. Overview of Existing Technologies
II. Initial Design Considerations
III. The Construct and its Implementation
IV. Current Status
V. Future Directions

Project Goal

To create a genetically addressable bacterial communication system

Project Goal



Addressable Conjugation vs. Chemical Communication: Advantages

- Rational design of separate specific communications channels

- Ability to transfer complex genetic information, instead of a single chemical signal

Addressable Conjugation vs. Chemical Communication: Disadvantages

- Slower
  - Conjugation ~ 8-18 hours
  - Chemical Means ~ 2-8 hours
- Conjugation occurs in clumps
  - Heterogeneity
  - Limited multiple usage

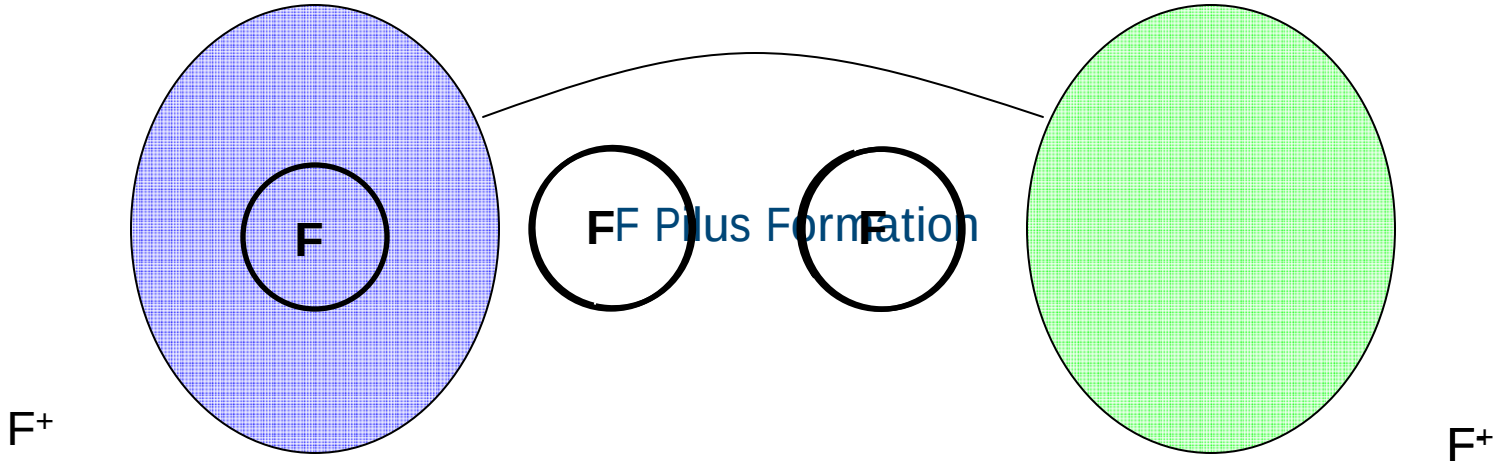
Implementation

Program: BioBricks System

Hardware: lambda-Red

Bacterial Conjugation

- Certain bacterial plasmids are classified as having a “fertility factor” i.e. F^+
- Cells that have a F^+ plasmid can conjugate and transfer their DNA to other bacteria



Choosing Conjugal Plasmids

There are many plasmids that are classified as conjugal. For our project, we used F and RP4 plasmids for the following reasons:

- F and RP4 exhibit differing pili lengths, biasing the order in which F and RP4 will conjugate
- F and RP4 do no conjugate with themselves
- F and RP4 are among the most studied and well-characterized conjugal plasmids
- F and RP4 plasmids are readily available

Important Facts about Conjugal plasmids

- Conjugal plasmids are very large, from 60k – 100k basepairs long => no standard cloning/transformation

- The TraJ protein is a regulatory protein responsible for initiating the DNA transfer cascade

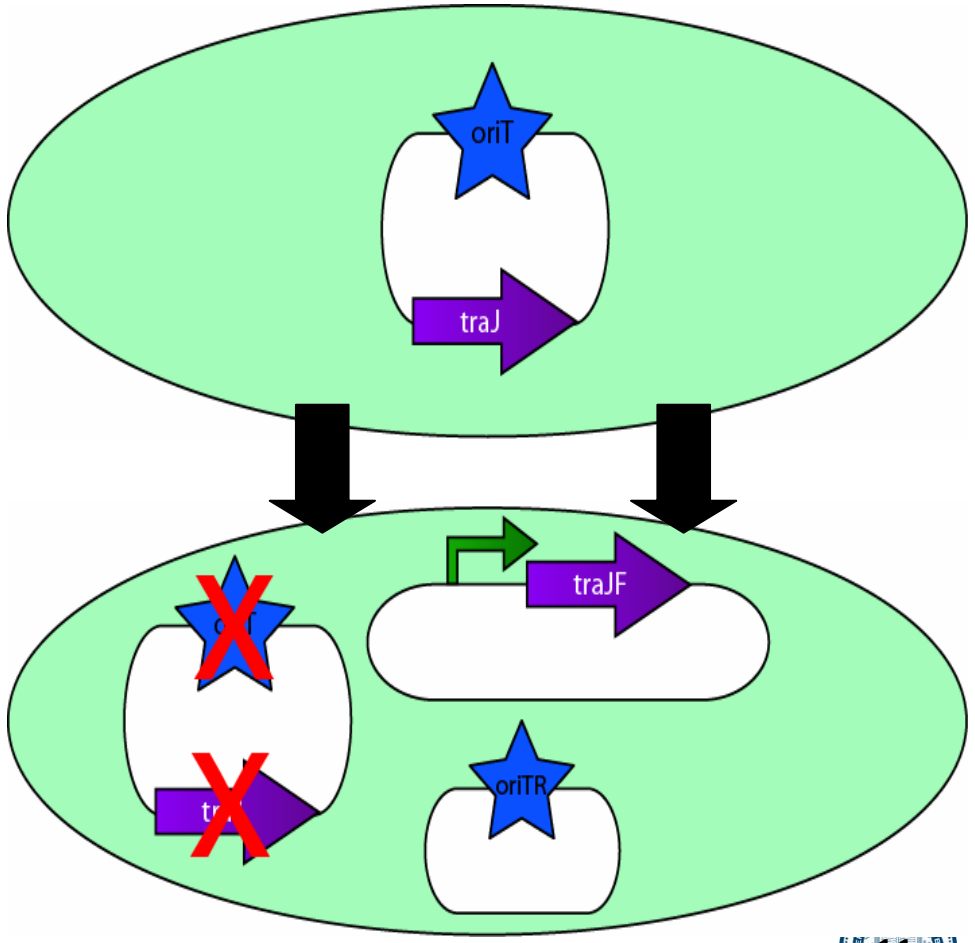
- DNA transfer during conjugation always begins at a specific sequence on the plasmid, OriT, the Origin of Transfer.

Modification of conjugative plasmids

- TraJ was cloned and placed into biobrick plasmids under the control of promoters of our choosing

- The OriT region was also cloned and placed into biobrick plasmids thus creating small, mobilizable plasmids

- The OriT region and TraJ gene were knocked out with Lambda-Red mediated recombination to prevent unwanted transfer of the F/R plasmid

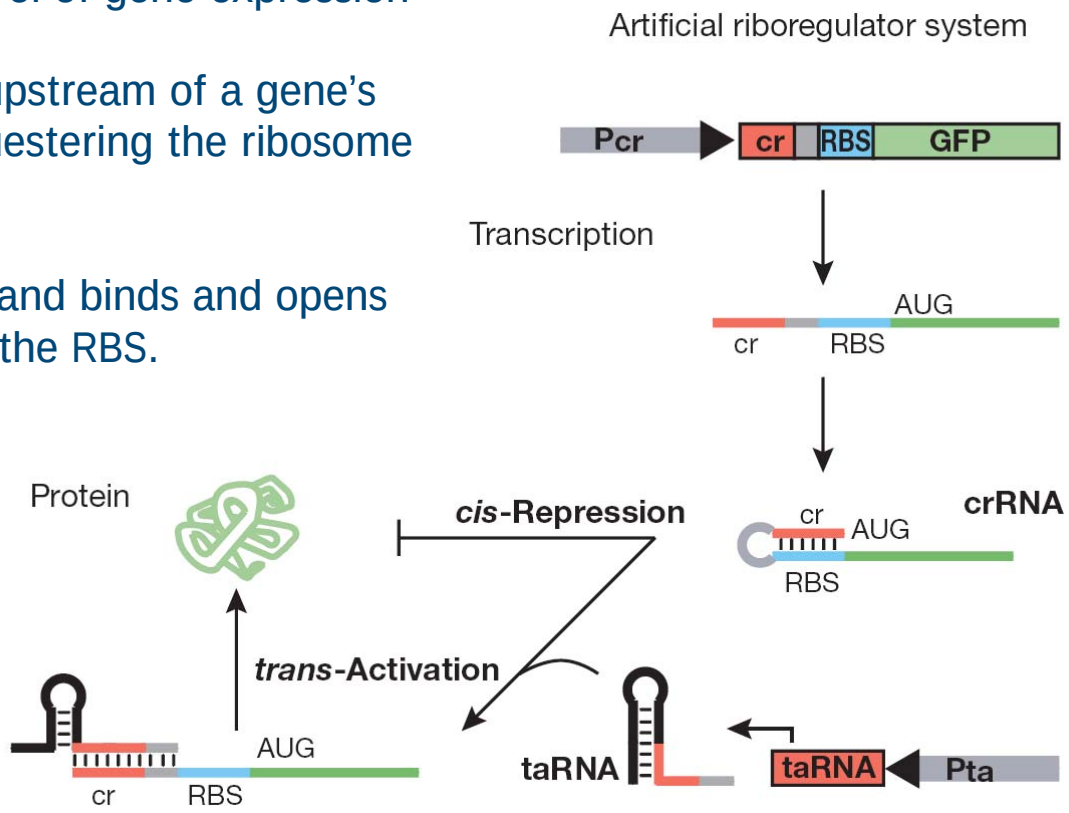


Conjugation Results

- An R-plasmid bearing cell can conjugate with an F-plasmid bearing cell
- The F plasmid and R-plasmid OriT knockouts fail to conjugate
- The OriT-R biobrick plasmid is mobilizable by the R-plasmid with OriT knocked out

The Riboregulator

- Method of postranscriptional control of gene expression
- *cis*-repressive sequence ("lock") upstream of a gene's coding region forms a hairpin, sequestering the ribosome binding site
- *trans*-activating ("key") mRNA strand binds and opens the hairpin thus allowing access to the RBS.
- Highly specific activation occurs. Very similar lock and key pair sequences do not exhibit crosstalk



Isaacs et al., *Nature Biotechnology,* 2004

Biobricked Riboregulator

- Tacking biobrick ends onto the end of the lock sequence would be ineffective due to the distance restrictions between a ribosome binding site and a gene's start codon
- The mixed site was thus incorporated directly downstream of the ribosome binding site
- The five base pair region between the hairpin loop and ribosome binding site was used as our address space to create two new lock sequences

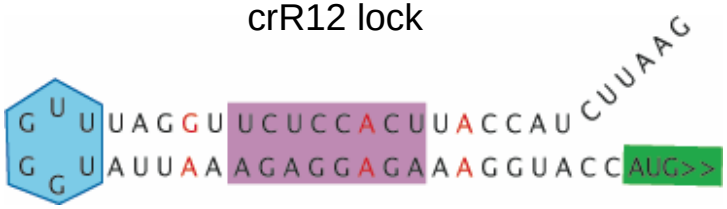
Lock from Isaacs Paper

Predicted mRNA structure of one of our Locks

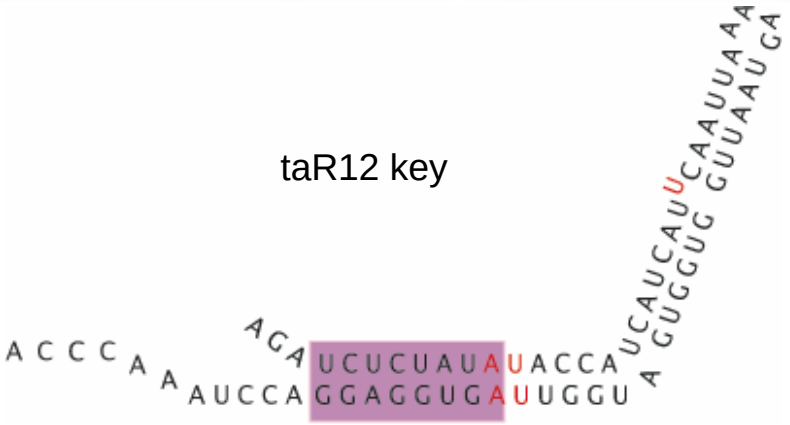
RBS region | Biobrick Mixed Site | Address Region | Hairpin loop | Start of locked gene

Biobricked Riboregulator

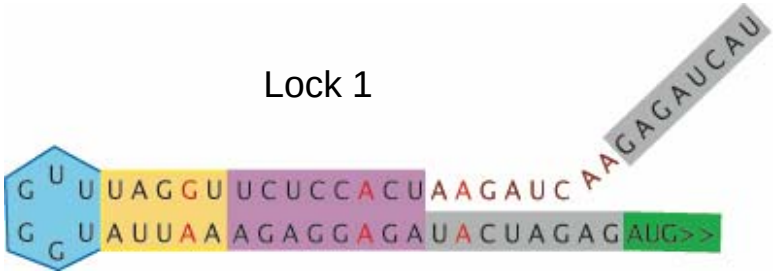
crR12 lock



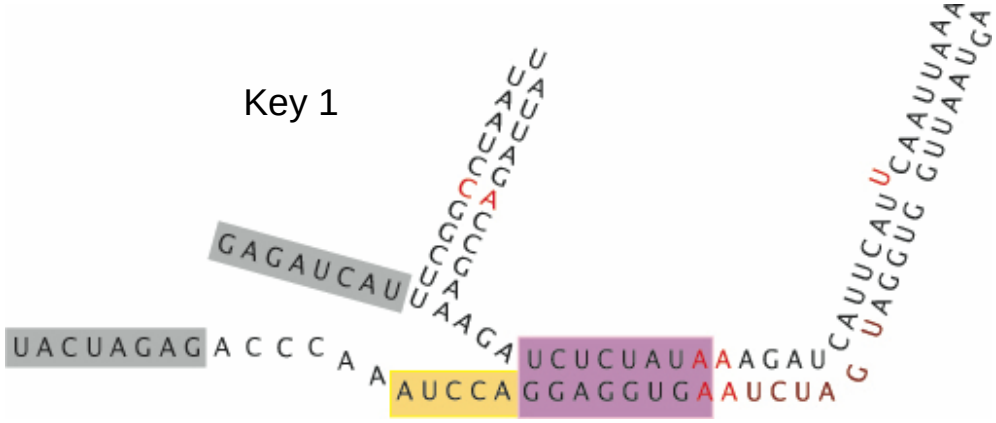
taR12 key



Lock 1



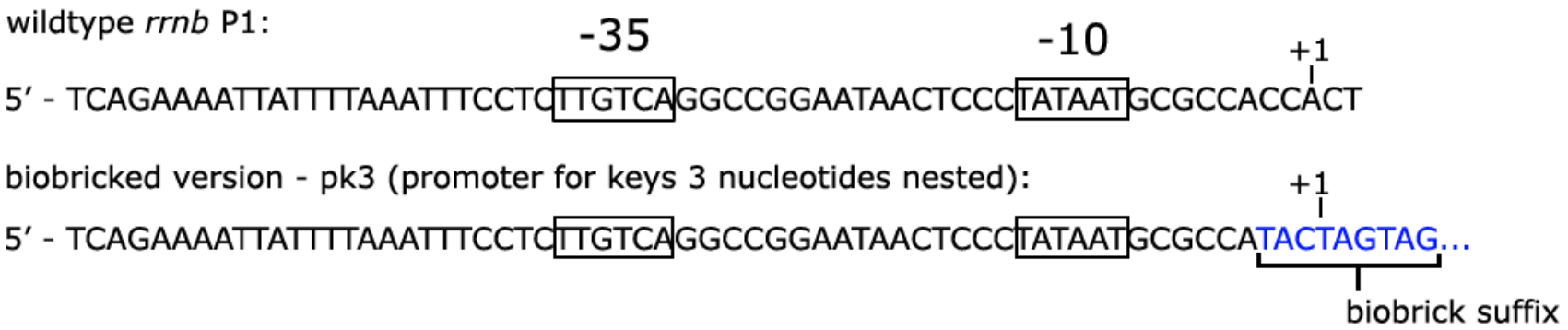
Key 1



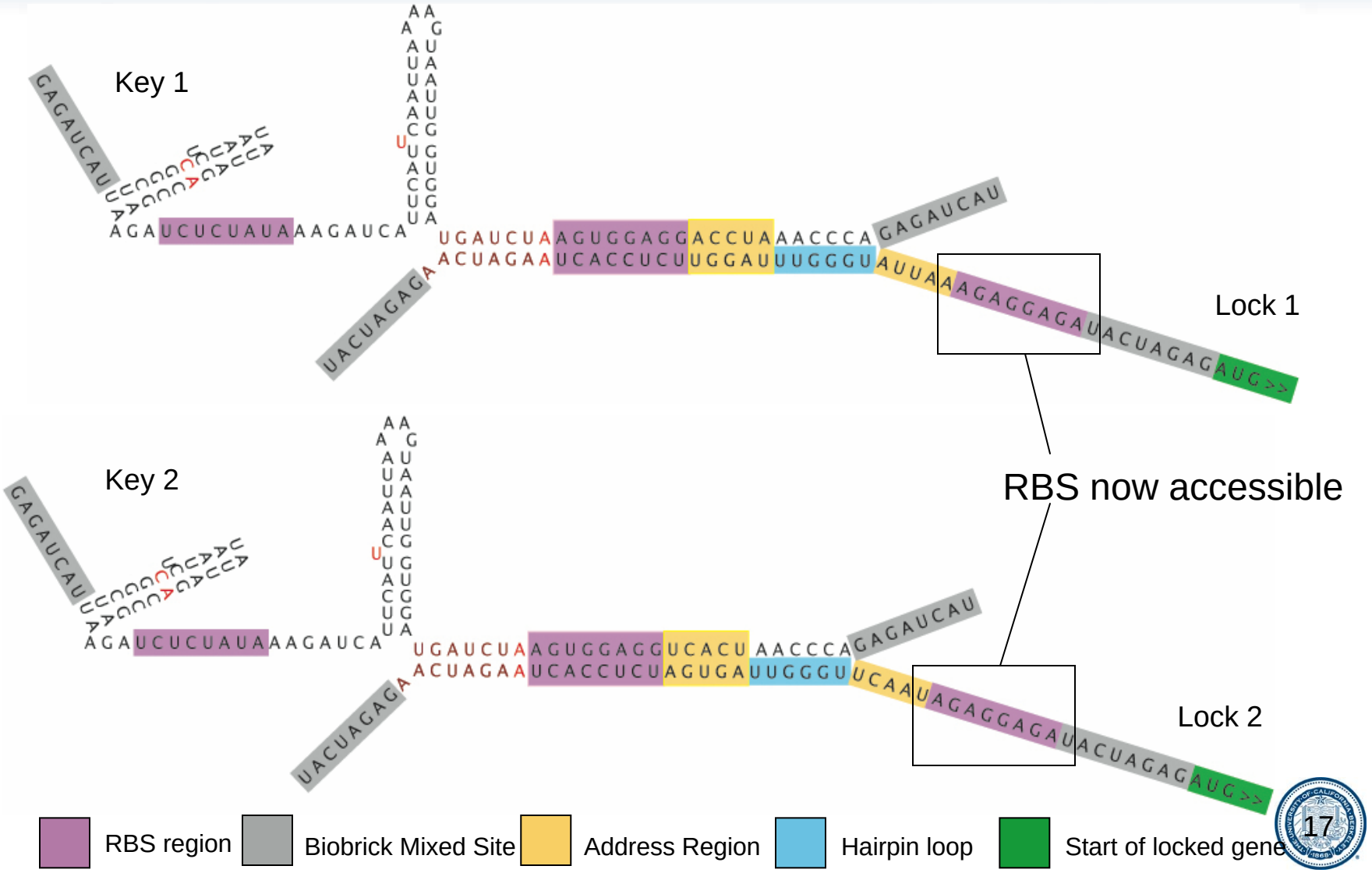
RBS region | Biobrick Mixed Site | Address Region | Hairpin loop | Start of locked gene

Biobricked Riboregulator

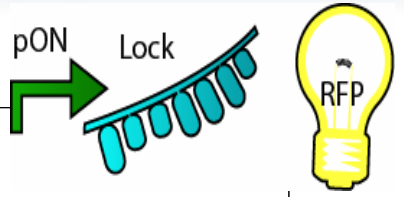
- Activation by the key sequences was highest when transcribed five nucleotides from the transcription start site *(Isaacs, et al.)*
- We created a biobricked derivative of the *E. Coli rrn*b P1 promoter to provide constitutive production of our keys
- Three nucleotides of the biobrick suffix were nested into the 5' end of the wildtype sequence in order to transcribe the keys at the desired five nucelotide distance.

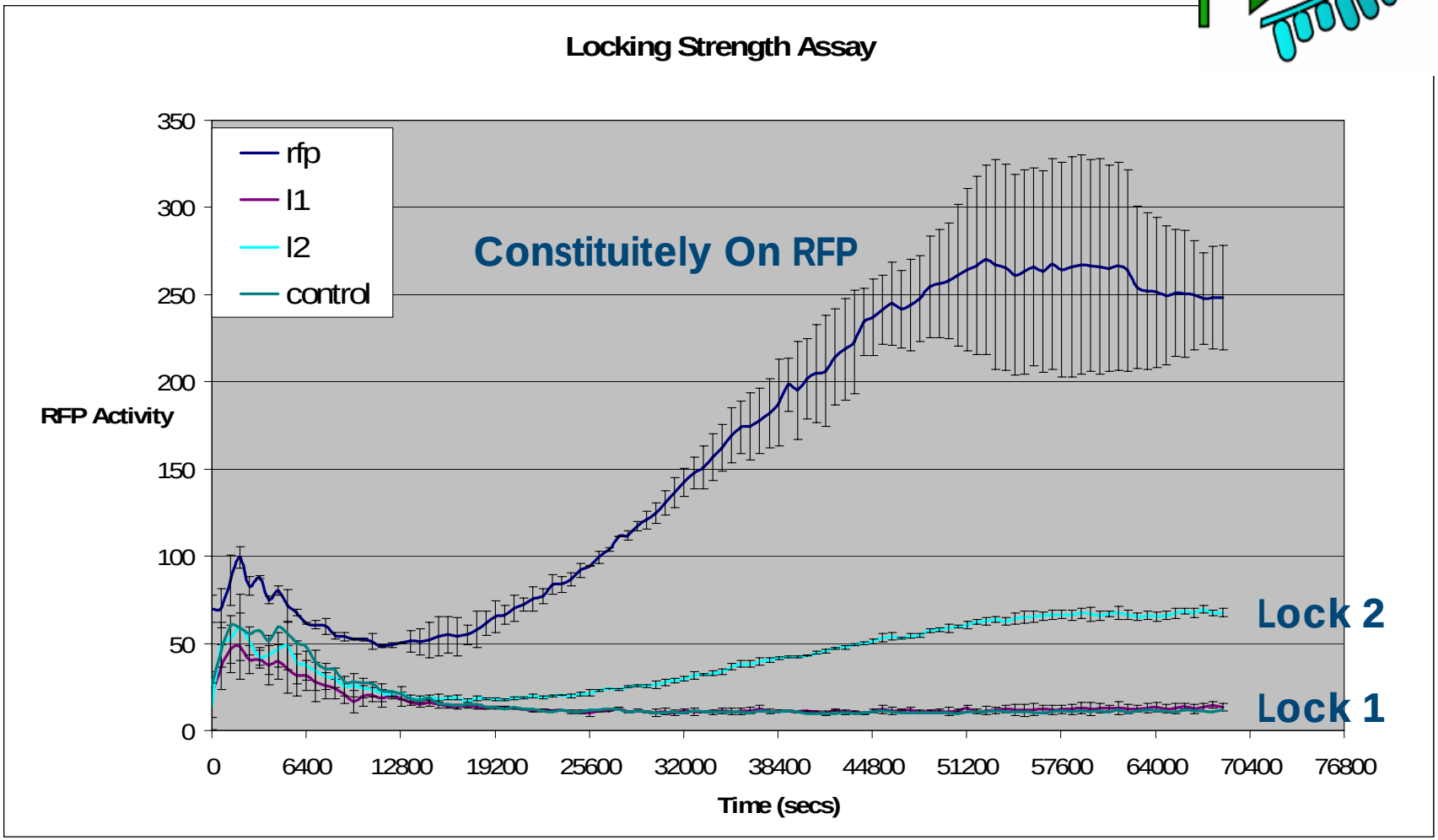


Unlocking the Riboregulator



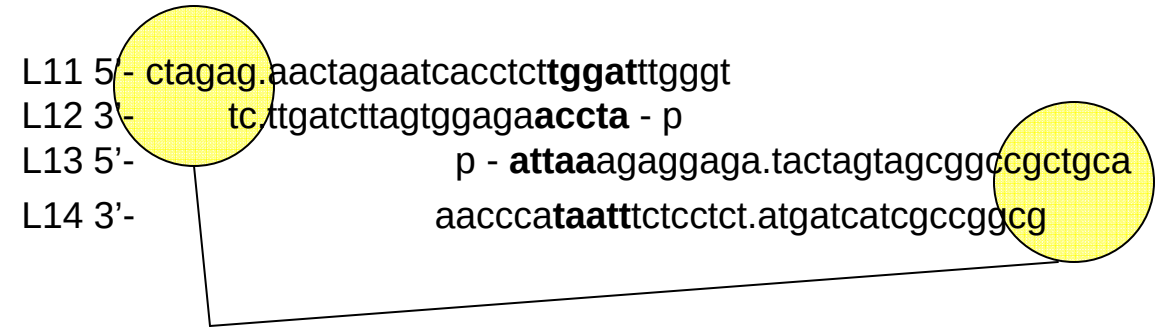
Biobricked Riboregulator





Riboregulator Construction

- Locks and keys are separated at hairpins into pairs of easily ordered oligos ~ 30 bp.
- One of each pair is ordered phosphorylated for easy ligation of annealed products
- Anneal pairs in separate tubes (heat to 95°C, unplug heatblock), combine, ligate.

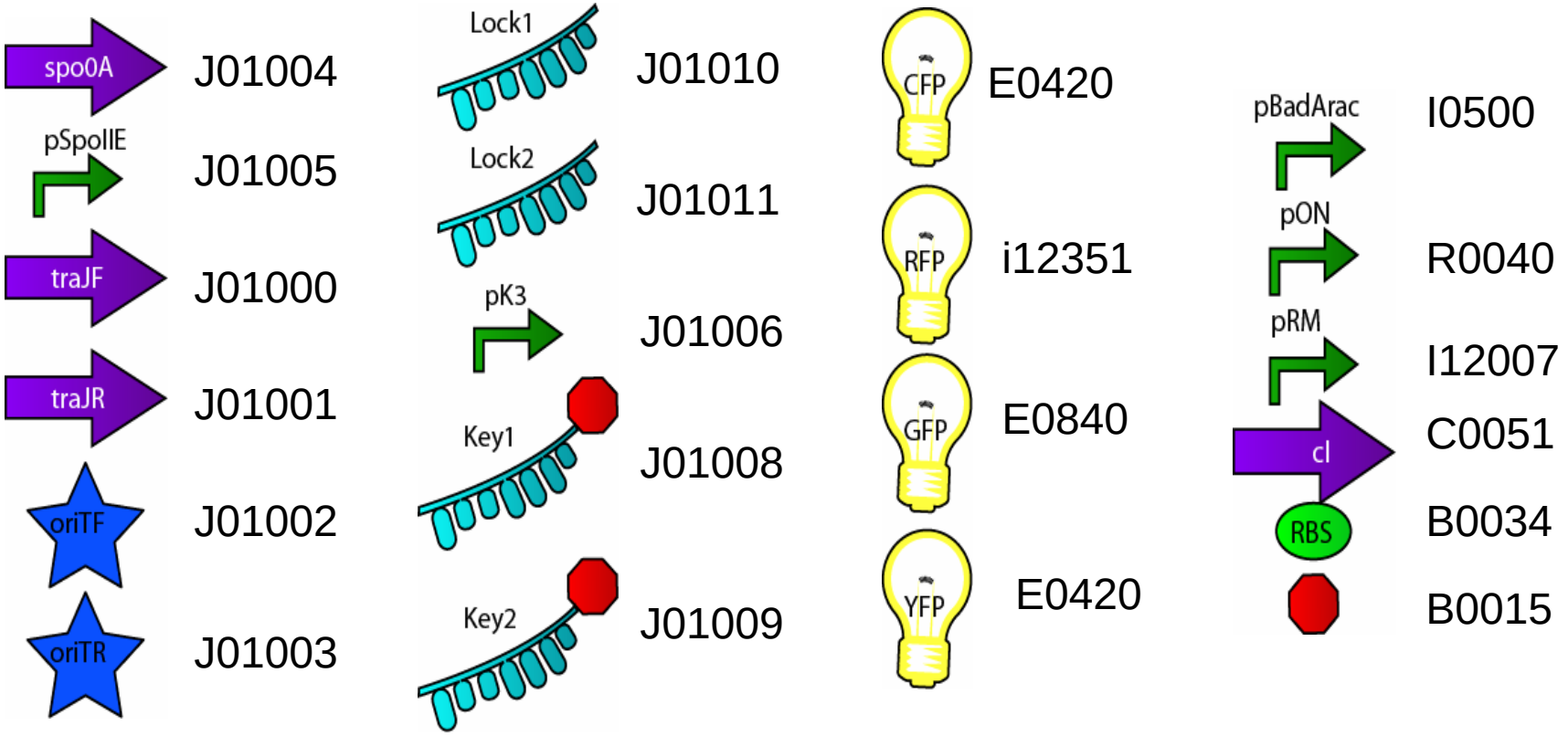


When annealed and ligated, result already has XbaI and PstI sticky ends…ready for assembly

- Keys require extra pair due to inclusion of key terminator (hairpin) within the part.

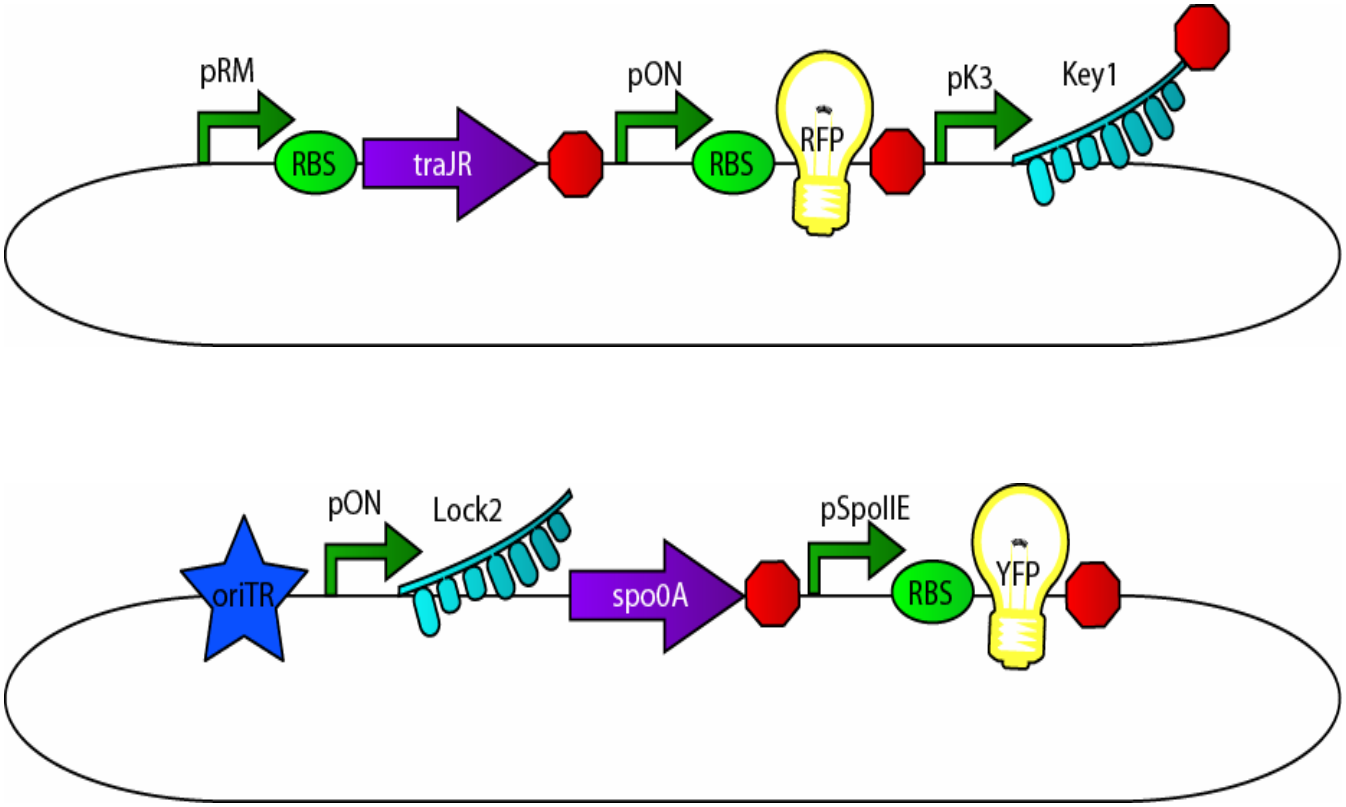
Construction

Parts Used


spo0A
J01004
pSpoIIE
J01005
traJF
J01000
traJR
J01001
oriTF
J01002
oriTR
J01003
Lock1
J01010
Lock2
J01011
pK3
J01006
Key1
J01008
Key2
J01009
CFP
E0420
RFP
i12351
GFP
E0840
YFP
E0420
pBadArac
I0500
pON
R0040
pRM
I12007
cI
C0051
RBS
B0034
B0015

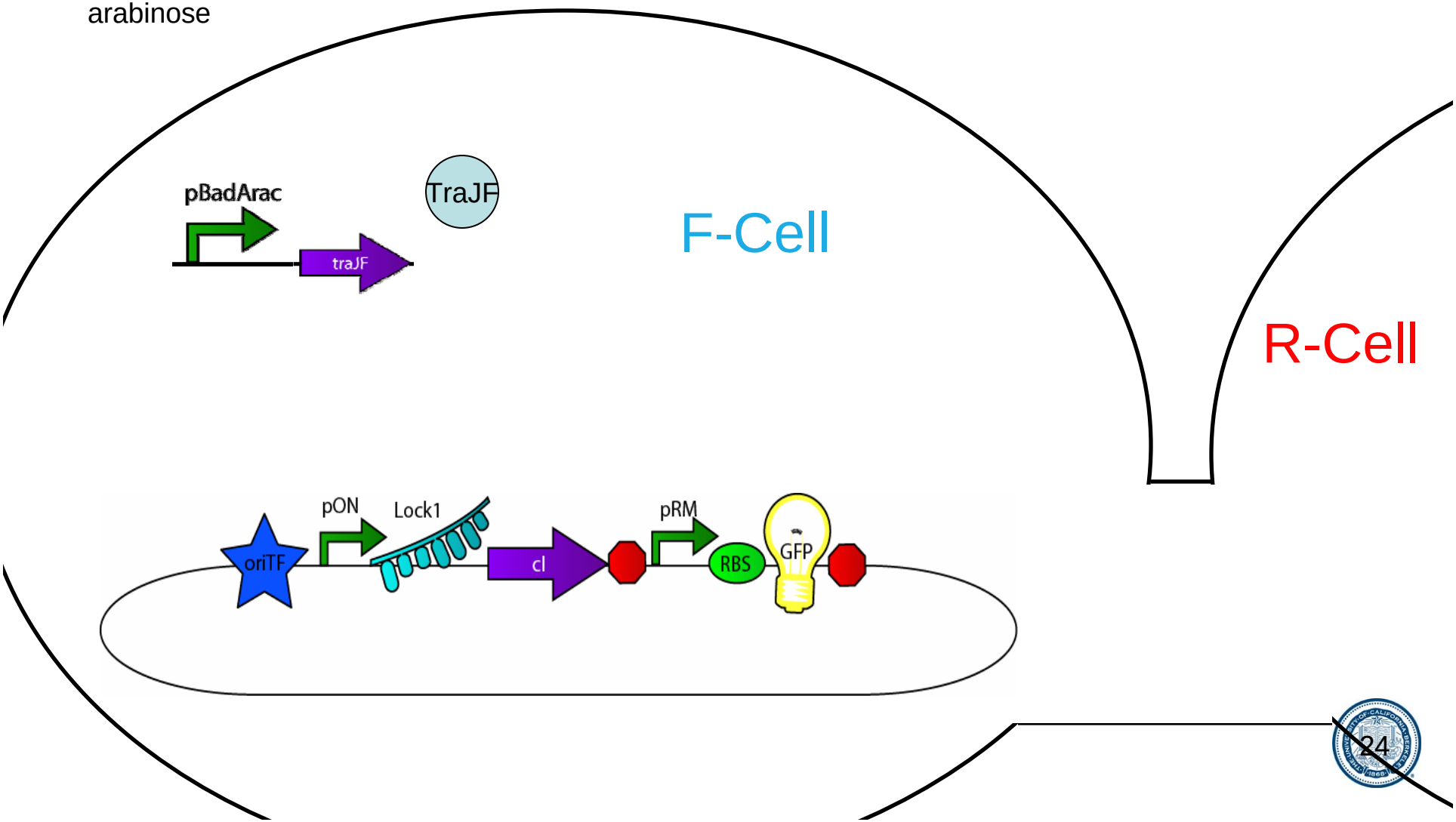
Construction Path

R-Cell Plasmids

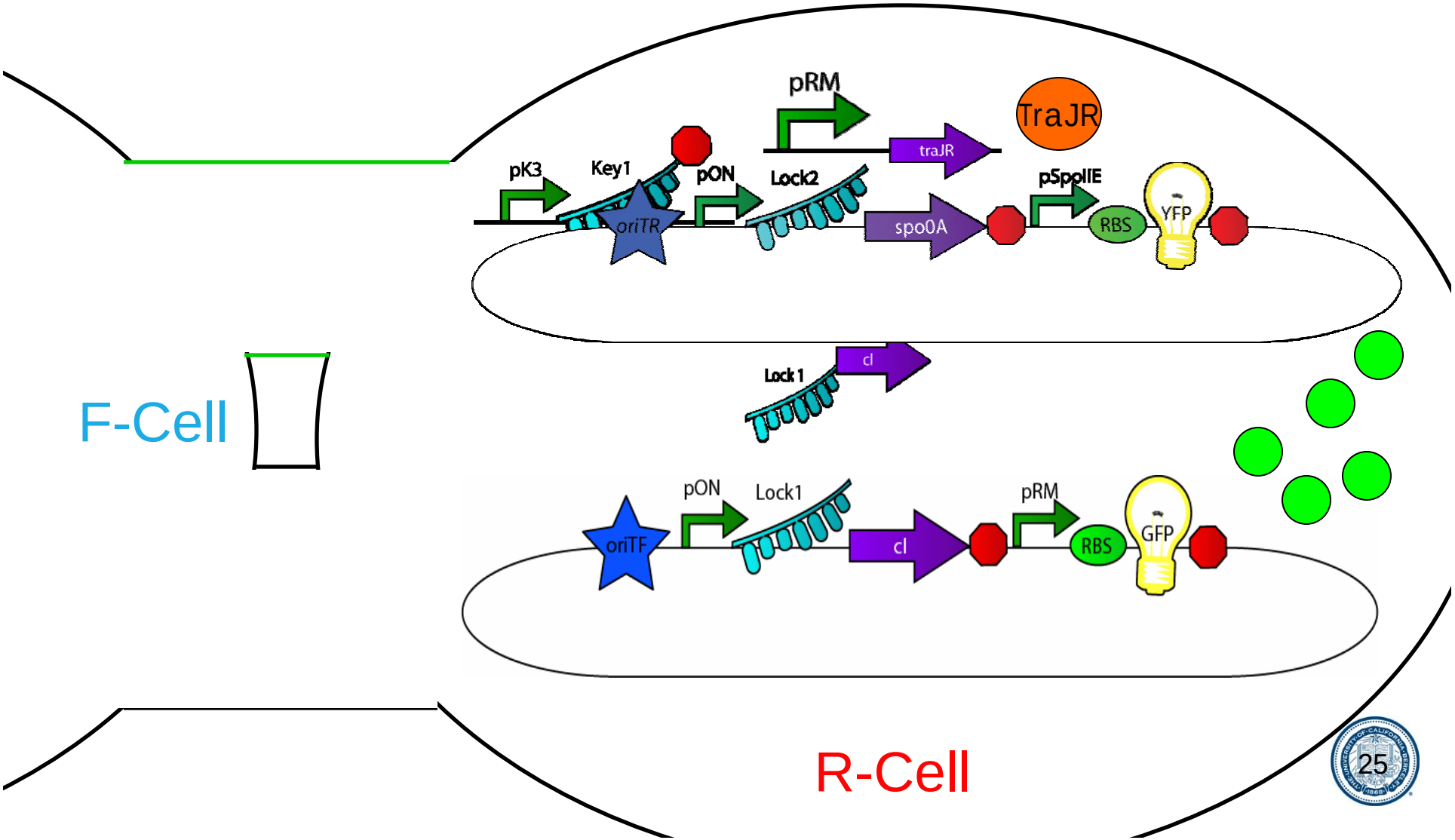


Sequence of Events

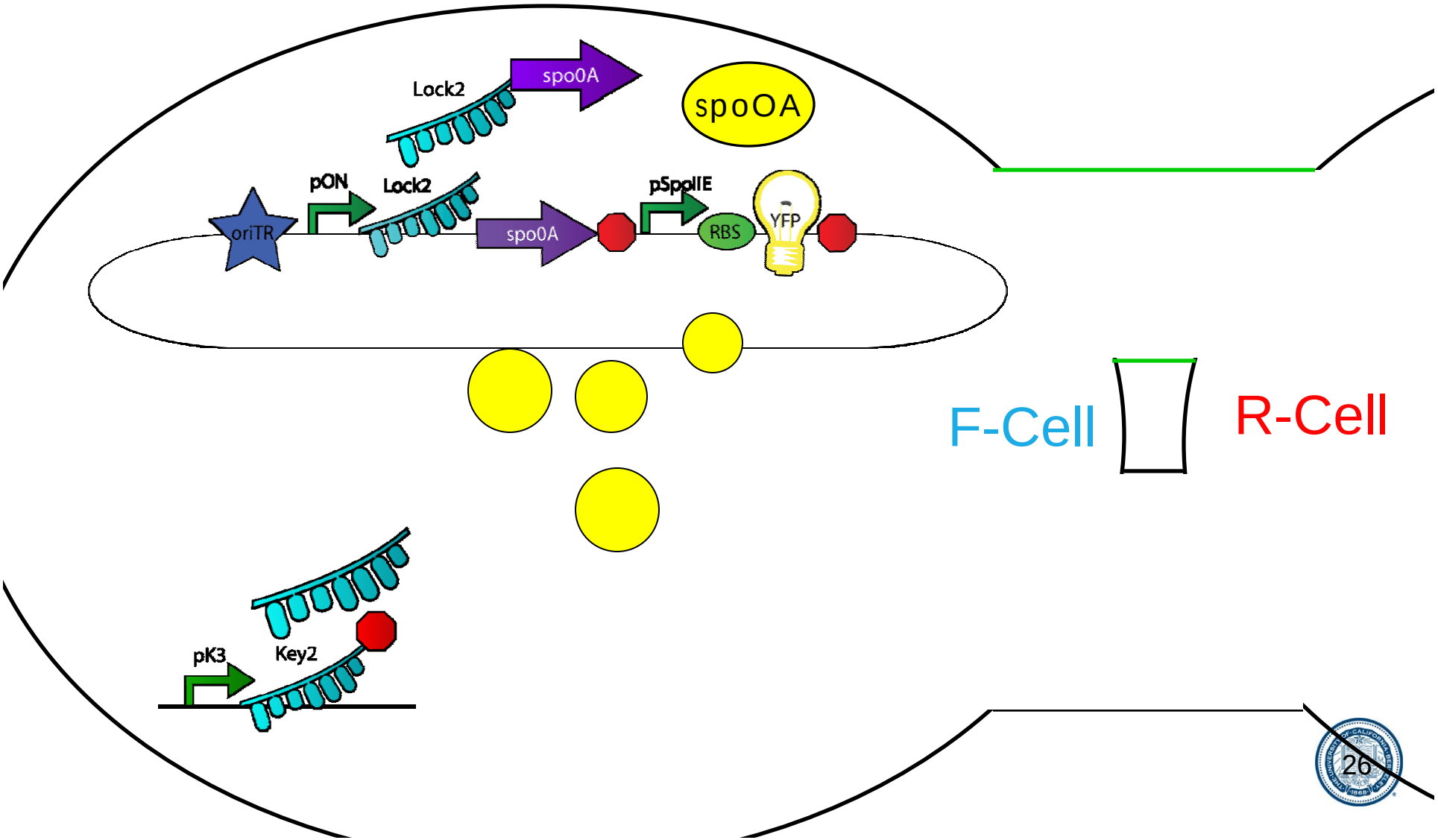
arabinose



Sequence of Events

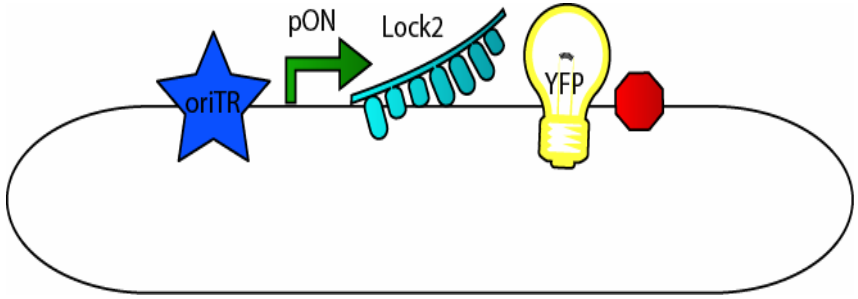


Sequence of Events

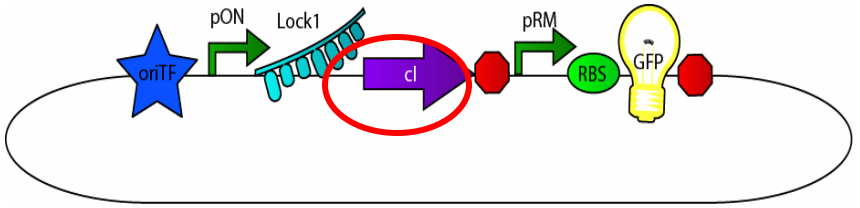


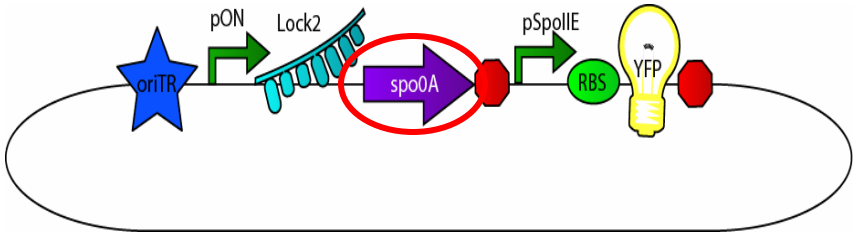
Modular Design

- Why didn't we just lock the fluorescent proteins?

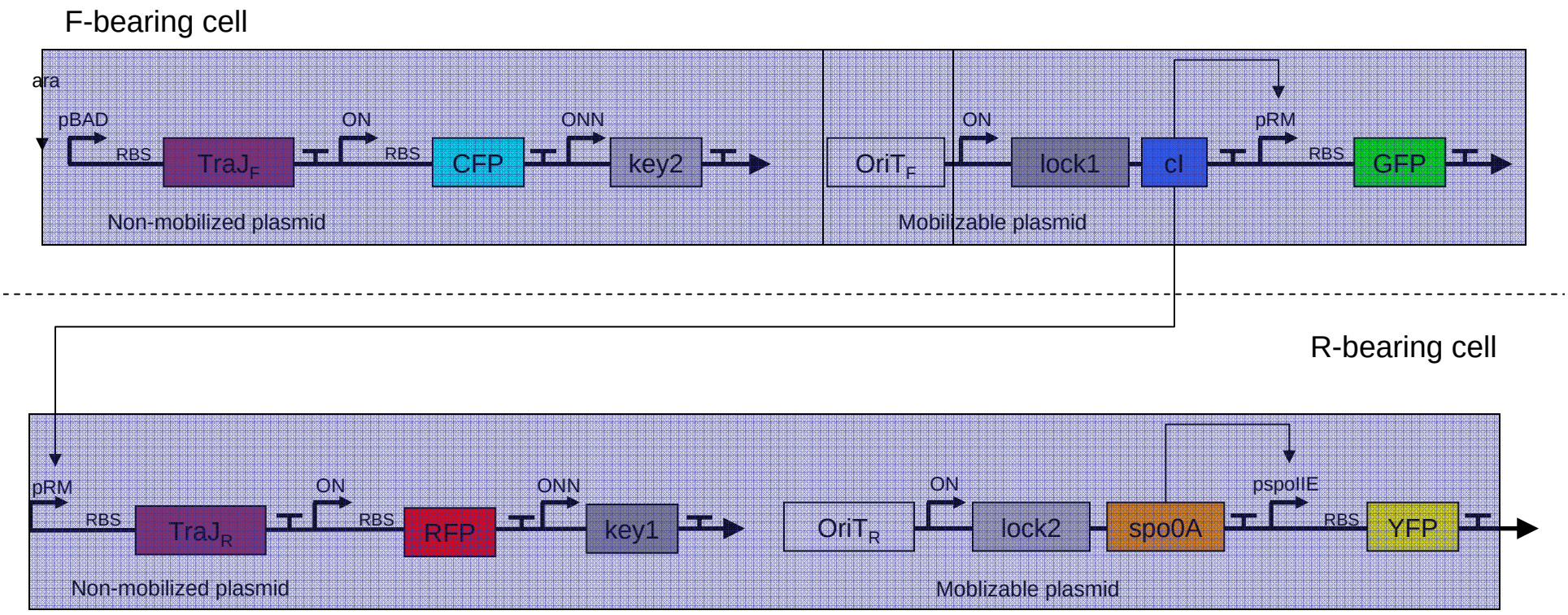


- Modularity and flexibility of design (send out inquiry for message verification!) with the addition of spoOA, cI signal





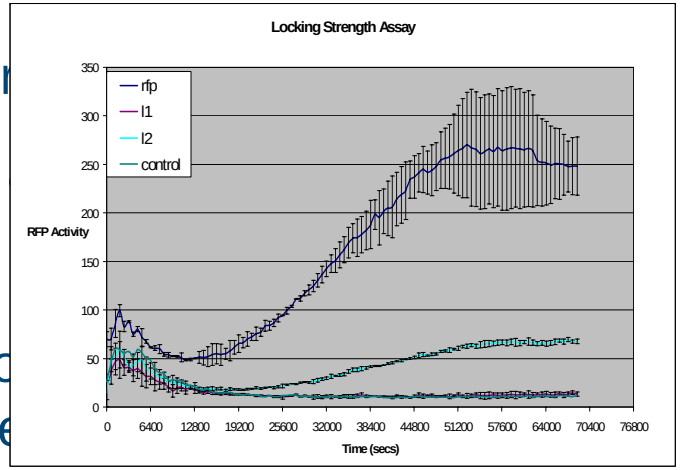
Progress thus far...



Implementation Issues

- Transferred plasmid croaks
- Slight leakiness of the lock we designed
  - Made several additional lock/key pai
  - Only one so far works (most are too le
  - Efficiency of conjugation is bad
  - OriT apparently not entirely knocked c

problem with labmda Red curing proce



Modest Goal

- Finish a one-way communication
  - Materials ready: 1 lock+key pair that works

- Test that the lock/key mechanism successfully can activate the program

- iGEM 2006?

Future Projects

- Two-way communication
- Extending address space

Berkeley iGem would like to thank the following people

Plasmid and Gene Providers

- Dr. Virginia Waters: RP4/RK2 plasmid
- Dr. Laura Frost: F-Plasmid
- Philip Silverman: pox38 F-Plasmid
- Dr. Farren Isaacs: Lock and Key Sequences
- Mike Cantor: SpoOA and pspollE plasmid

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