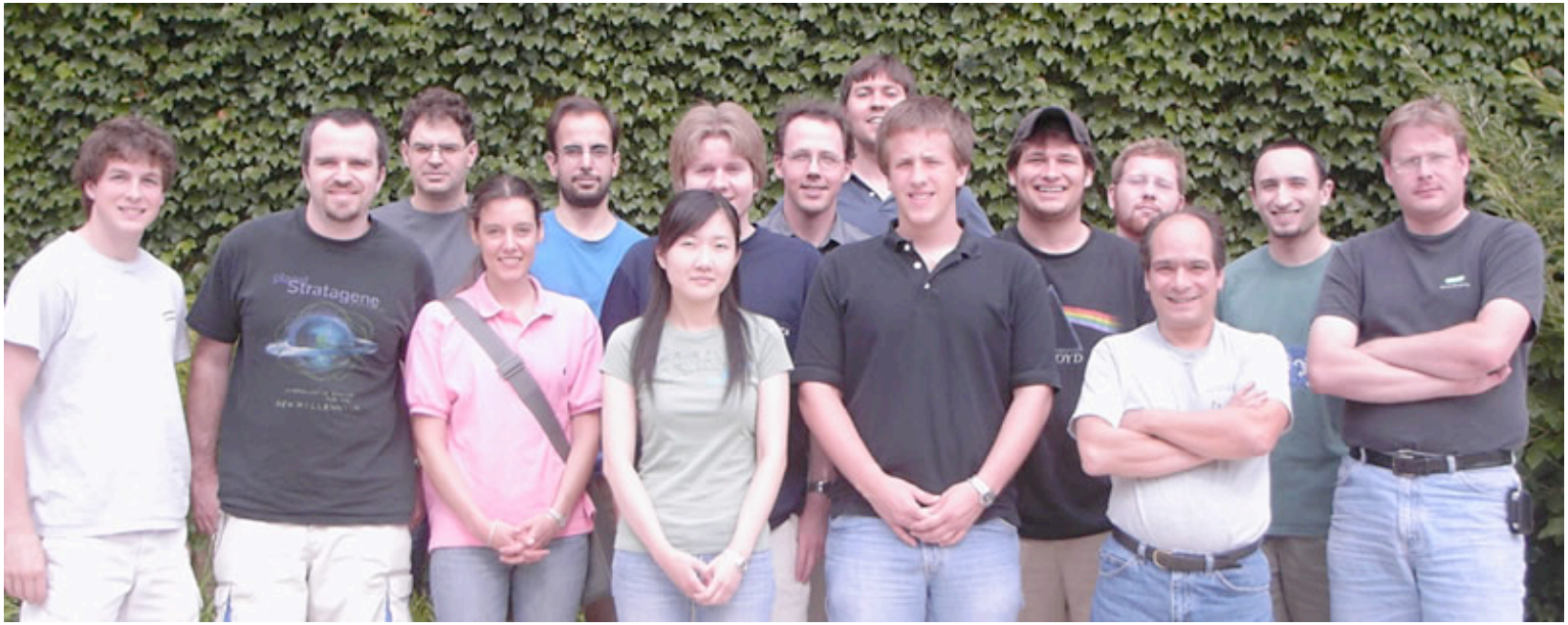
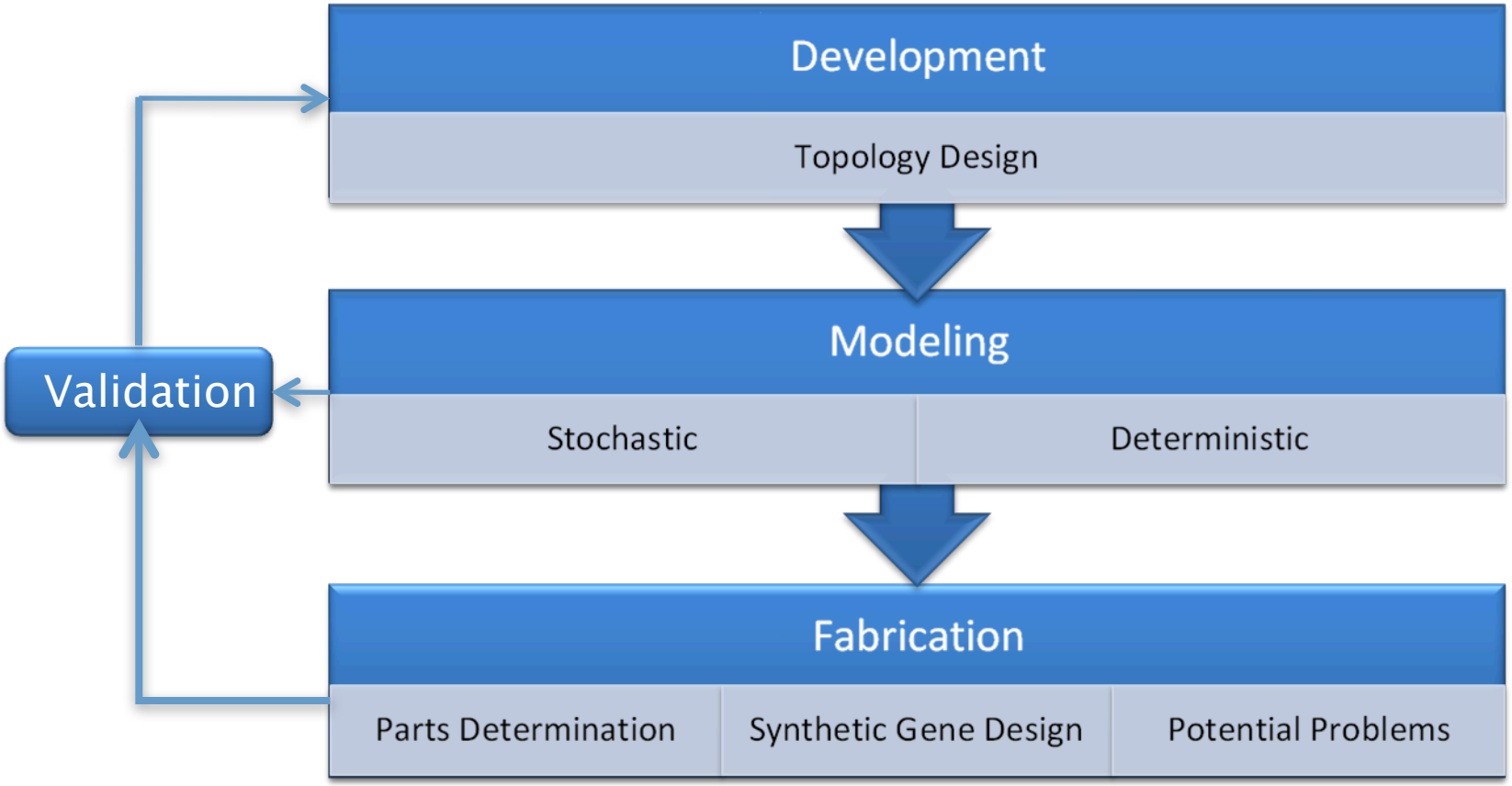
University of Michigan iGem Team

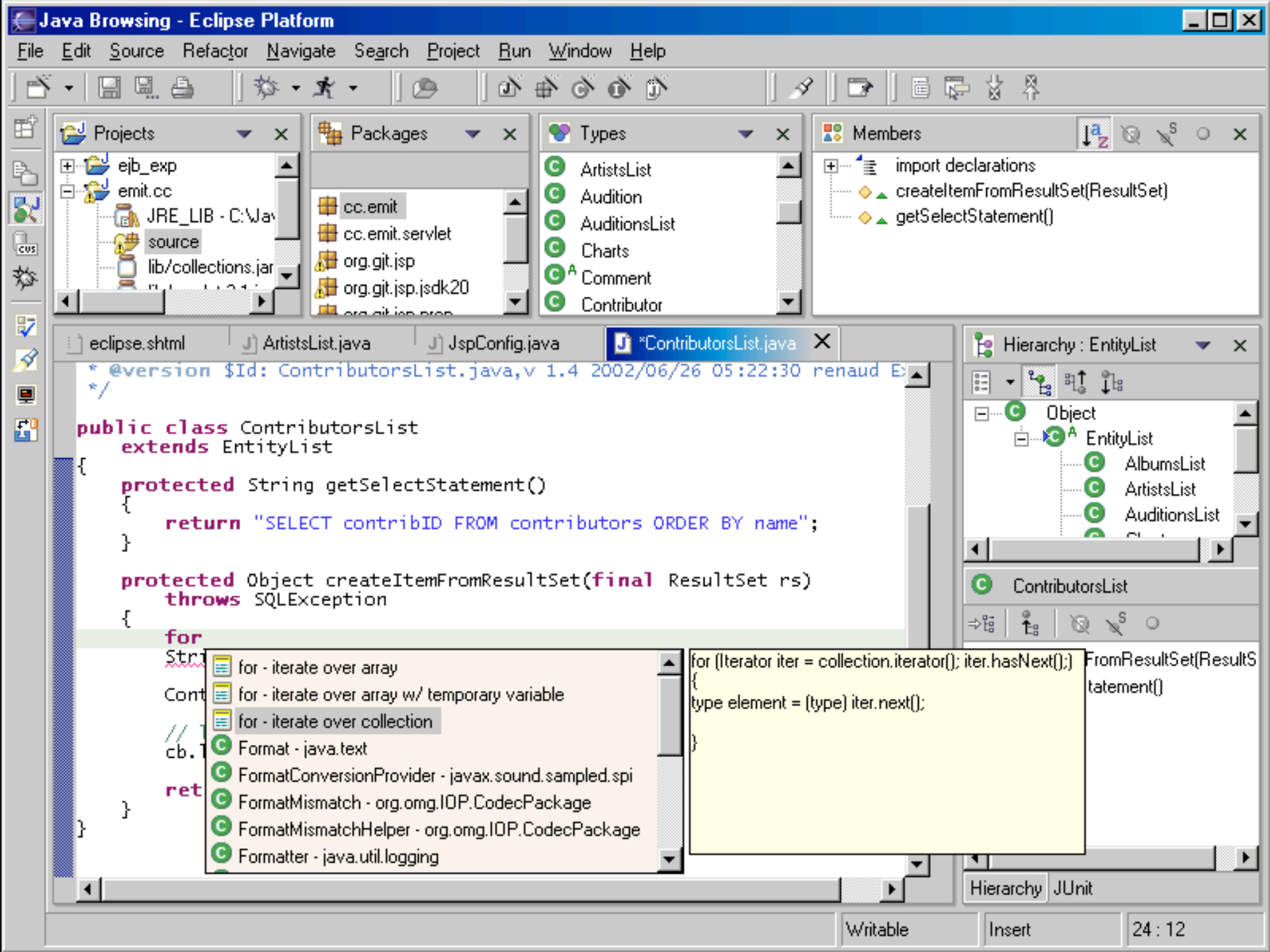


Synthetic Biology Pipeline



Plasmene

Rapid, Computer-Aided Biological Network Design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File Edit Source Refactor Navigate Search Project Run Window Help
Projects
ejb_exp
emit.cc
JRE_LIB - C:\Jav
source
lib/collections.jar
Packages
cc.emit
cc.emit.servlet
org.gjt.jsp
org.gjt.jsp.jsdk20
Types
ArtistsList
Audition
AuditionsList
Charts
Comment
Contributor
Members
import declarations
createItemFromResultSet(ResultSet)
getSelectStatement()
eclipse.shtml
ArtistsList.java
JspConfig.java
*ContributorsList.java
* @version $Id: ContributorsList.java,v 1.4 2002/06/26 05:22:30 renaud E
*/
public class ContributorsList
extends EntityList
{
protected String getSelectStatement()
{
return "SELECT contribID FROM contributors ORDER BY name";
}
protected Object createItemFromResultSet(final ResultSet rs)
throws SQLException
{
for
for - iterate over array
for - iterate over array w/ temporary variable
for - iterate over collection
Format - java.text
FormatConversionProvider - javax.sound.sampled.spi
FormatMismatch - org.omg.IOP.CodecPackage
FormatMismatchHelper - org.omg.IOP.CodecPackage
Formatter - java.util.logging
for (Iterator iter = collection.iterator(); iter.hasNext();)
{
type element = (type) iter.next();
}
Hierarchy : EntityList
Object
EntityList
AlbumsList
ArtistsList
AuditionsList
ContributorsList
Hierarchy
JUnit
Writable
Insert
24 : 12

Plasmene

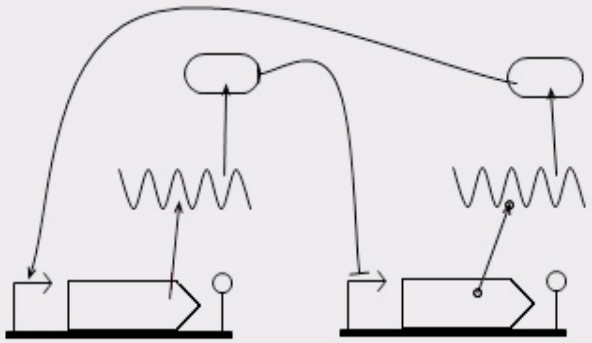
File Edit Model SBW Help

Parts

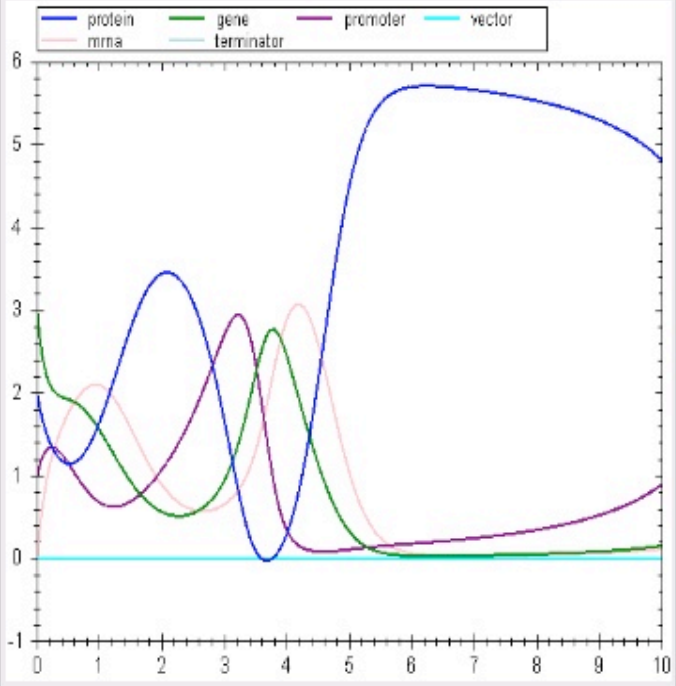
Commonly Used | Generics | Promoters | Genes | mRna's | Proteins | Terminators | Vectors | Compounds

Vector Promoter Gene Terminator mRNA Protein

New Topology | Clock V3



New Topology Modelling Results | Clock V3 Modelling Results



ProjectExplorer

- Model: Oscillators
  - Parts
    - Promoter 1
    - LacZ Gene
    - LacZYA Promter
    - General Terminator
    - LacY Gene
    - LacA Gene
    - E. Coli
    - Pseudomonas
    - GlnK Promoter
  - Topologies
    - Repressilator
      - Version 1
      - Weak Genetic Repression
      - Strong Genetic Repression
      - Anti-sense

Properties

Miscellaneous	
Name	**LacZ Transcription**
Description	**The reaction represents the tran**
Reaction Specific	
Reactants	**Reactants of this reaction**
Gene	**β-galactosidase monomer**
Type	**Gene**
Sequence	**ATGACCATGATTACGGATTCACTG**
Compartmer	**Cytosol**
Name	**β-galactosidase monomer**
Description	**The lacZ gene is one of the struc**
Products	Products of this Reaction
Protein	**β-galacotsidase**
KineticLaw	**k_transcription*LacZ_Gene**

Protein

Protein,RWSDGSYLEDRWSDGSYLEDRWSDGSYLED,β-galac otsidase is a metalloenzyme exhibiting broad substrate …

Miscellaneous

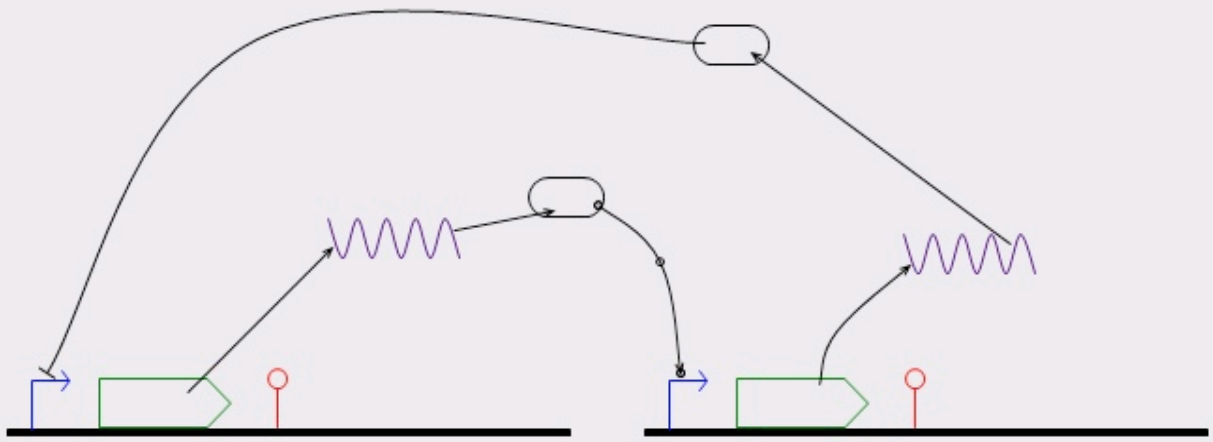
Properties | Plasmid | Host | Jobs

Number	Name	Component	Progress	Initiated	Completed	St
	Check for critical program updates.	Core	0%			Co
	Download Sda1 data from Ecocyc.	Data Wareh...	0%			In
	Retrieve sequence information from regul...	Data Wareh...	0%			Qu
	Perform restriction site analysis on E. Coli...	Analysis	0%			Qu
	Backup custom networks.	Data Wareh...	0%			Ca

Plasmene - Version 0.1

File Edit Add Part Project Modeling Fabrication Export Help

To SBML L1V1



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DefiningPoints	
EndPoint	{X=385, Y=255}
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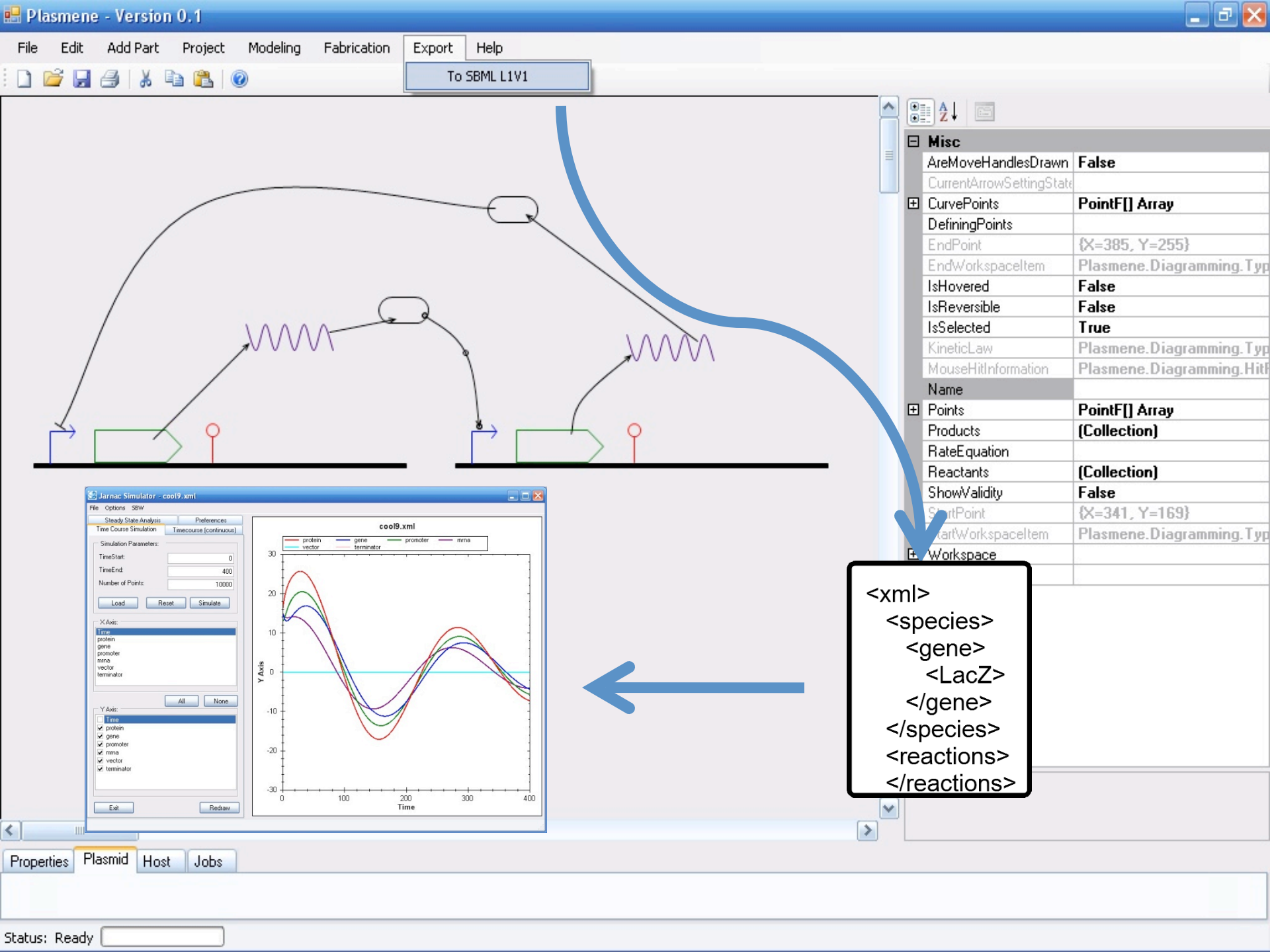
IsHovered	False
IsReversible	False
IsSelected	True
KineticLaw	Plasmene.Diagramming.Typ
MouseHitInformation	Plasmene.Diagramming.Hit
Name	
Points	PointF[] Array
Products	(Collection)
RateEquation	
Reactants	(Collection)
ShowValidity	False
StartPoint	{X=341, Y=169}
StartWorkspaceItem	Plasmene.Diagramming.Typ
Workspace	
WorkspaceLayer	

Name

Properties Plasmid Host Jobs

Status: Ready


Plasmene - Version 0.1
File
Edit
Add Part
Project
Modeling
Fabrication
Export
Help
To SBML L1V1
Misc
AreMoveHandlesDrawn
False
CurvePoints
PointF[] Array
DefiningPoints
EndPoint
{X=385, Y=255}
EndWorkspaceItem
Plasmene.Diagramming.Typ
IsHovered
False
IsReversible
False
IsSelected
True
KineticLaw
Plasmene.Diagramming.Typ
MouseHitInformation
Plasmene.Diagramming.Hit
Name
Points
PointF[] Array
Products
(Collection)
RateEquation
Reactants
(Collection)
ShowValidity
False
Workspace
Jarnac Simulator - cool9.xml
cool9.xml
protein
gene
promoter
mrna
vector
terminator
Time
Y Axis
<xml>
<species>
<gene>
<LacZ>
</gene>
</species>
<reactions>
</reactions>
Properties
Plasmid
Host
Jobs
Status: Ready

Technical Details

Work on Version 2 has begun.

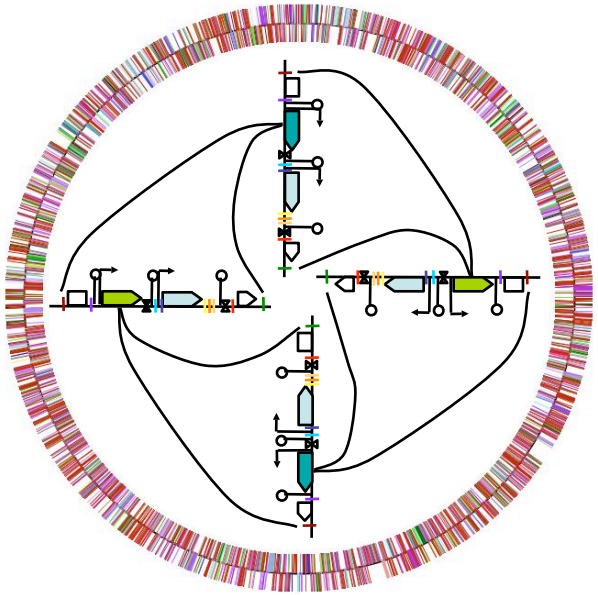
- Developed in C#
- Uses a variation of the model view presenter design pattern
- Currently composed of two independent projects – a diagramming component and a biology class library
- Communication project is planned to allow easy integration with websites, web services, and databases

Proof-of-concept version available for download from Michigan's page on the iGEM wiki.

Interested?

Contact Paul Rogero
ptrog@umich.edu

A System for Fabrication of Chromosomal Genetic Circuits by Nested Landing Pads



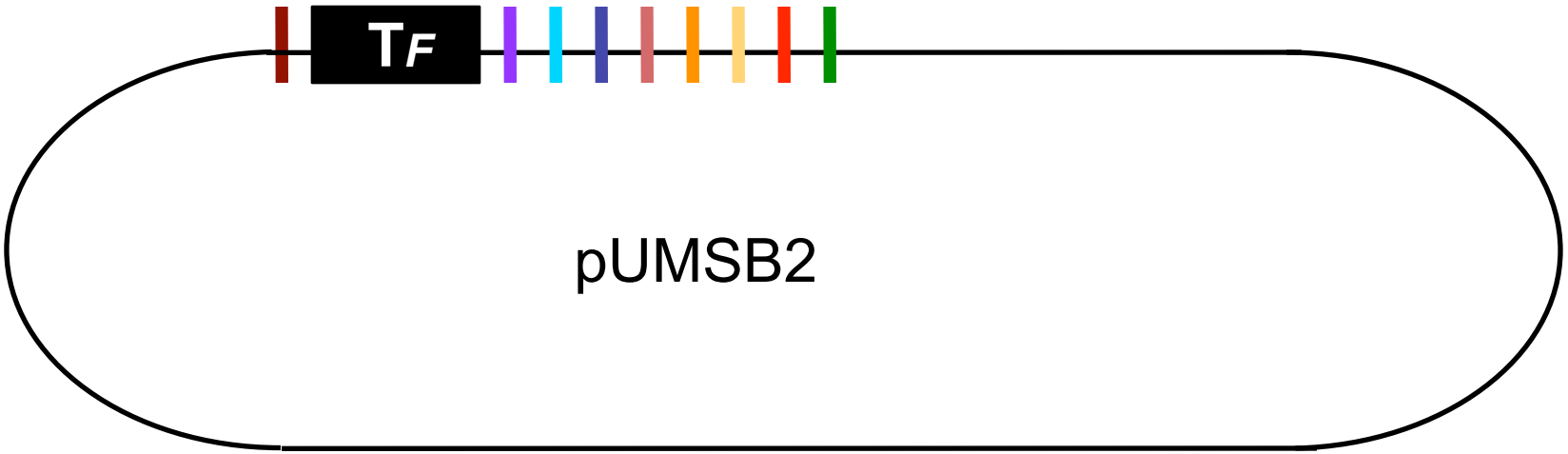
Why Use Our Chromosomal Landing Pads?

- Limit Noise
- Genetically Stable
- Simple phenotypic screening and genetic confirmation
- User-friendly construction

Landing Pad Construction

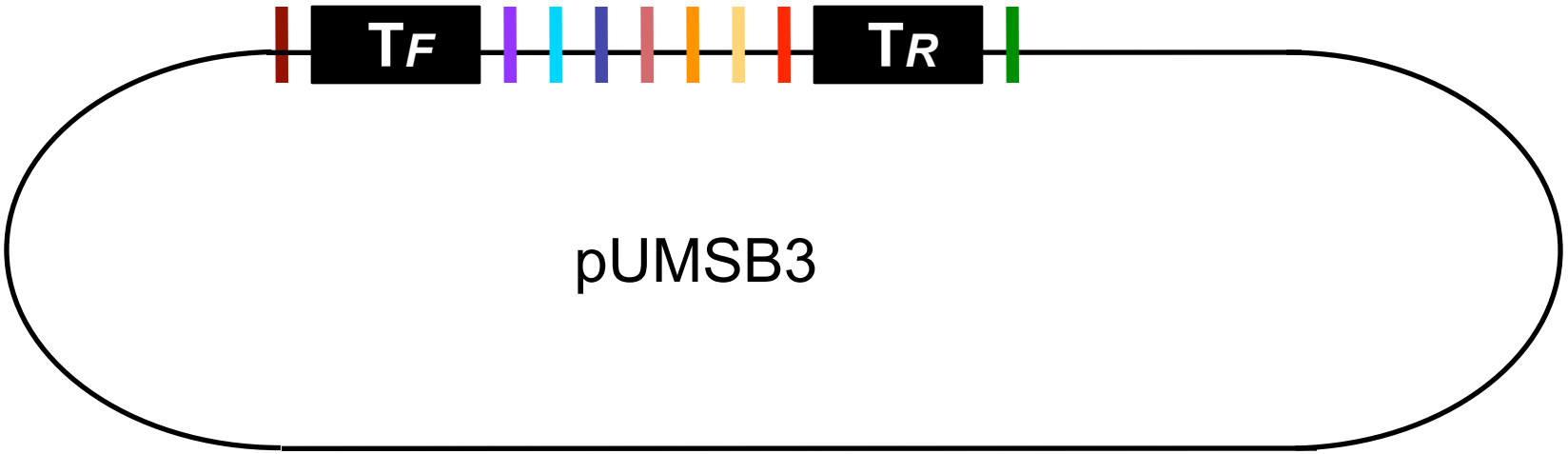
Polylinker: EcoRI, SacI, NotI, NdeI, ClaI, BamHI, SalI, XhoI, HindIII

Landing Pad Construction



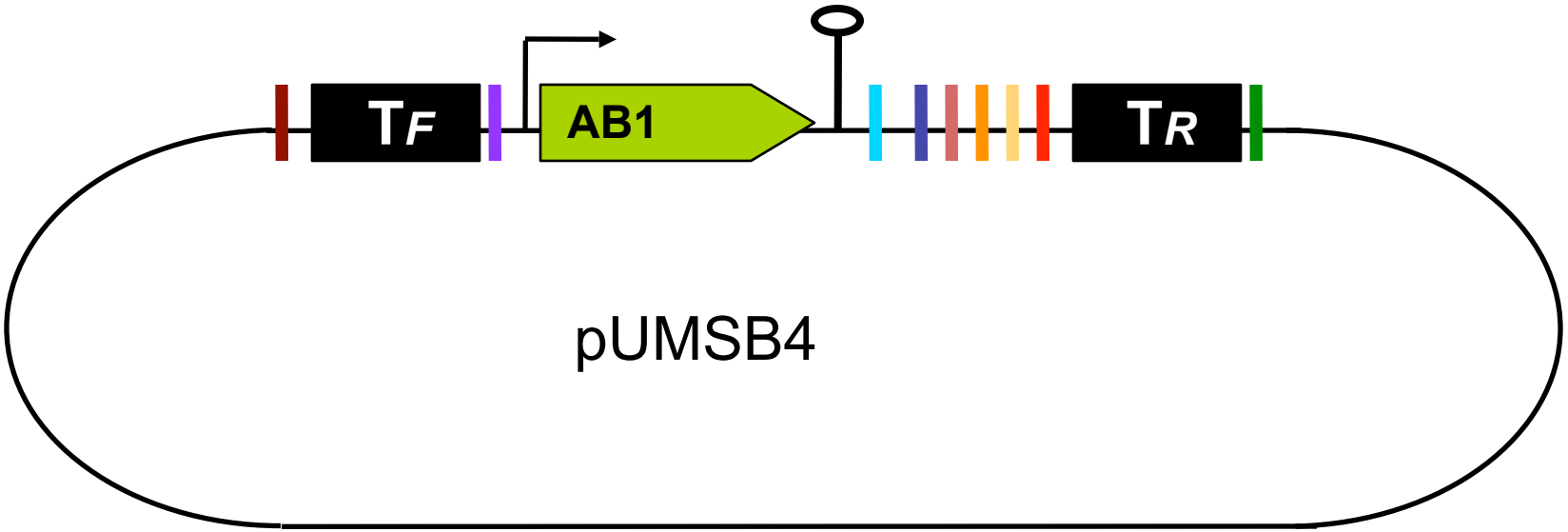
Polylinker: EcoRI, SacI, NotI, NdeI, ClaI, BamHI, SalI, XhoI, HindIII
T*F* - Front end of target gene

Landing Pad Construction



Polylinker: EcoRI, SacI, NotI, NdeI, ClaI, BamHI, SalI, XhoI, HindIII
T*R* - Rear end of target gene

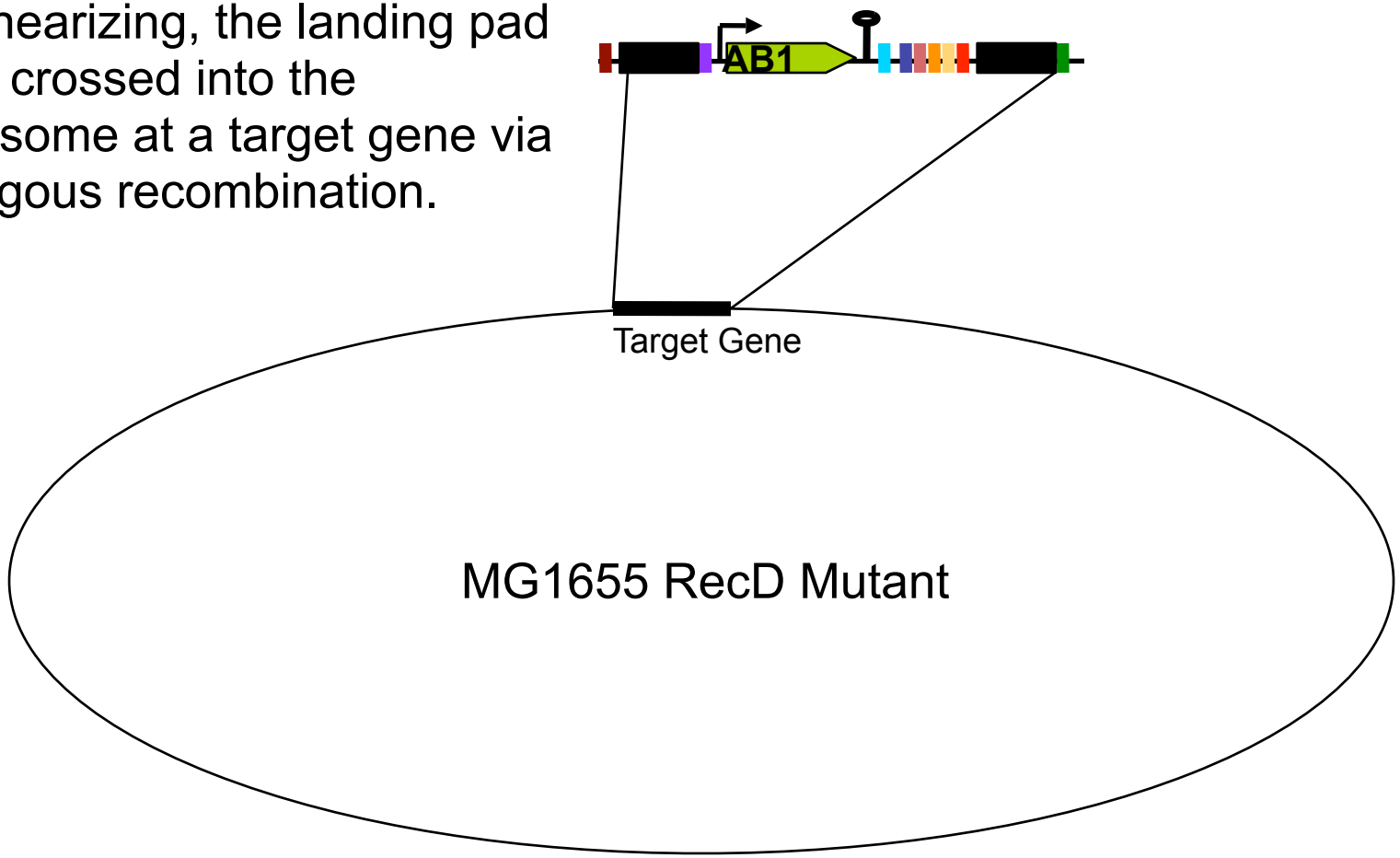
Landing Pad Construction



Polylinker: EcoRI, SacI, NotI, NdeI, ClaI, BamHI, SalI, XhoI, HindIII
AB1 - Antibiotic Resistance

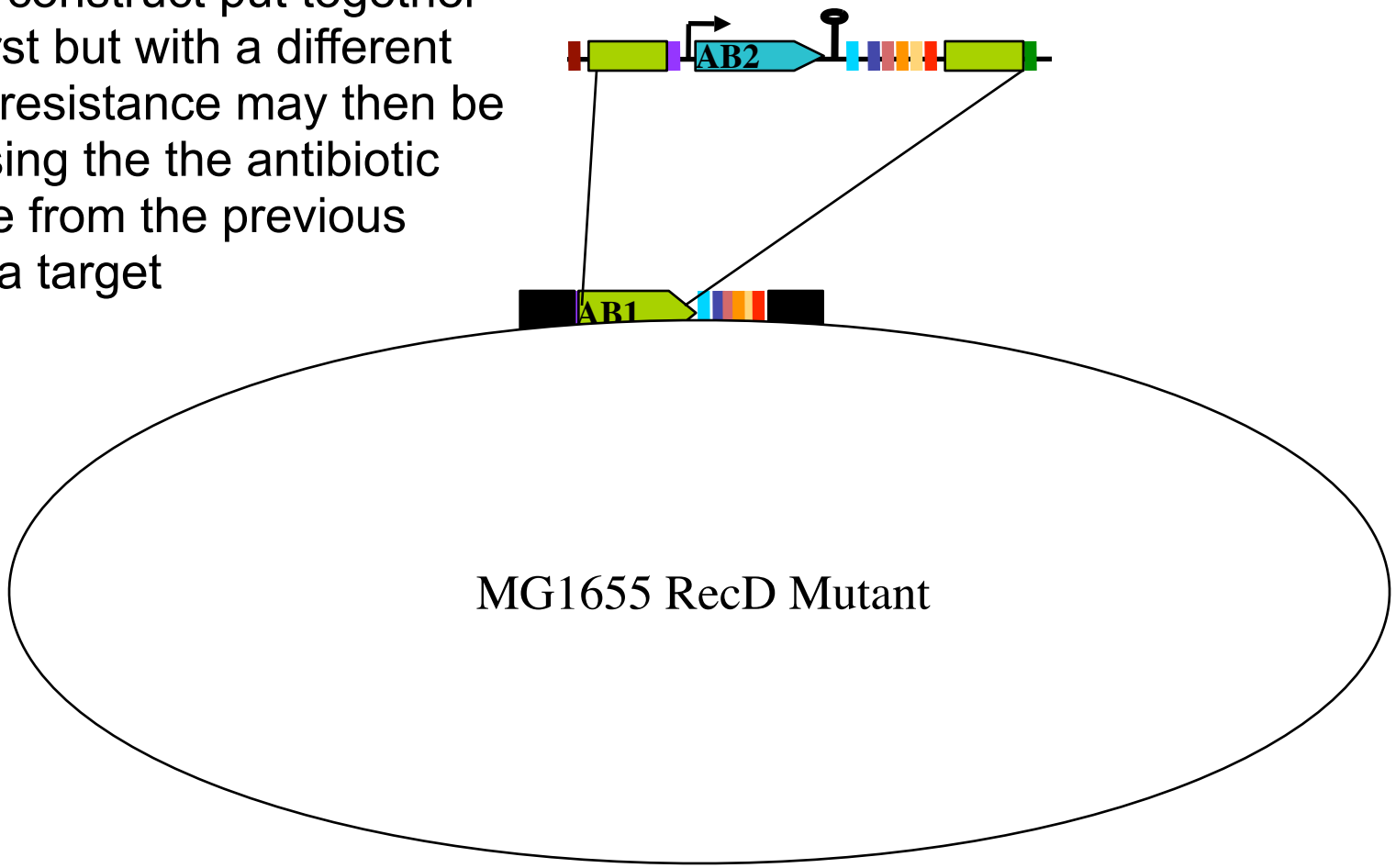
Homologous Recombination onto the Chromosome

Upon linearizing, the landing pad may be crossed into the chromosome at a target gene via homologous recombination.



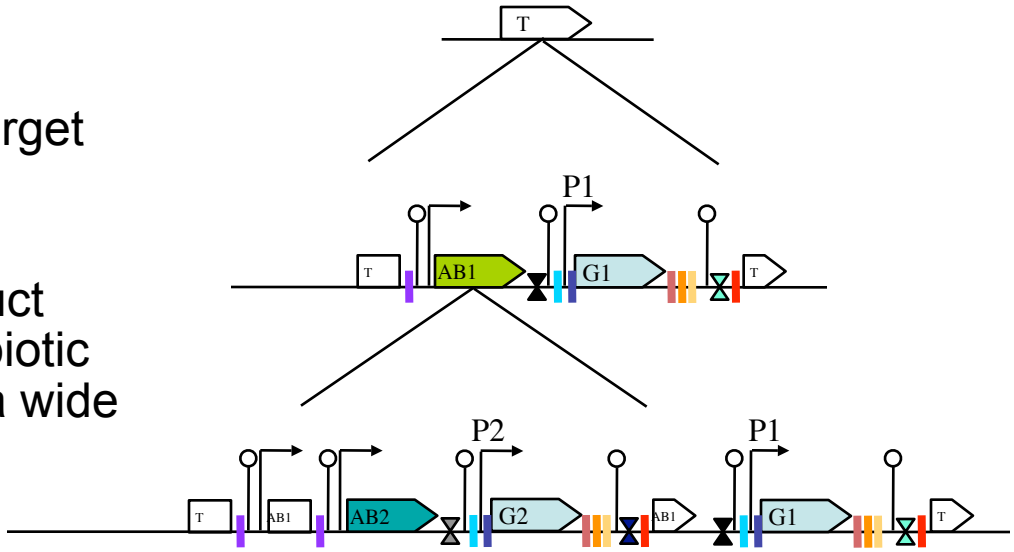
Homologous Recombination onto the Chromosome

A second construct put together like the first but with a different antibiotic resistance may then be nested using the the antibiotic resistance from the previous round as a target



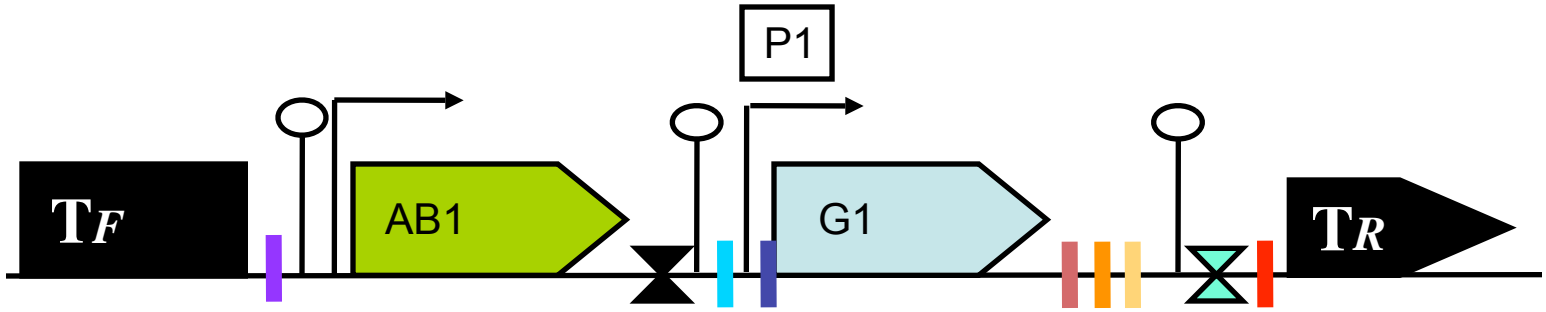
Unique Features

- Nesting
  - AB1 may be used as a target for future rounds of recombination.
  - By targeting AB1, construct essentially switches antibiotic sensitivities allowing for a wide range of constructs.



- Bar Codes (⧖)
  - –Unique 21 bp sequences placed at the end of each landing pad
  - –Allows for easy sequencing to check fidelity of recombinant techniques

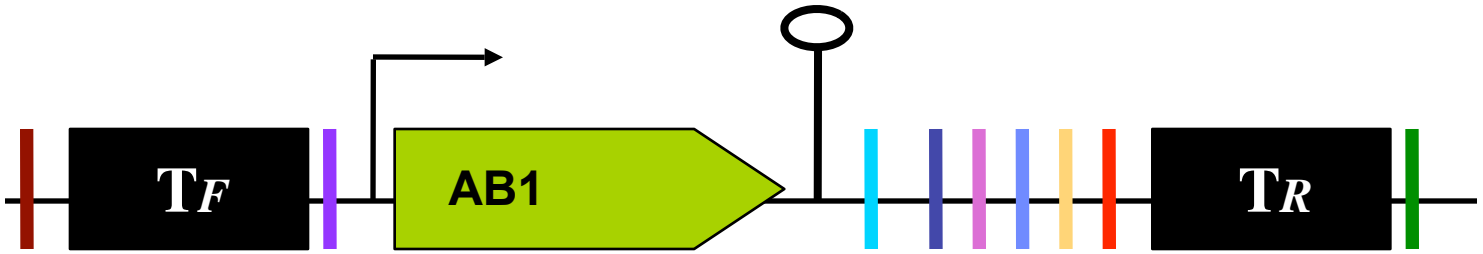
Custom Construction



- The design allows the experimentalist to mix and match custom promoter / gene / terminator combinations.

- A series of extra restriction sites within the polylinker gives flexibility to those parts which may be already constructed.

BioBrick Compatibility



- To make the current design BioBrick compatible, we reverse engineered a “patch” between the NotI and BamHI which contains both the XbaI and a SpeI site.

- Thus BioBricks may be built to designers desire as they normally would and then transferred into the landing pad.

Tuning Kinetic Order Through The Use Of Repressor Sinks

What is Kinetic Order?

Consider:

$$\frac{dy}{dx} = k_1 \frac{x^n}{k_2 + x^n}$$

What is Kinetic Order?

Consider:

What is Kinetic Order?

Consider:

Hill coefficient

What is Kinetic Order?

Consider:

Hill coefficient

(Hill coefficient describes kinetic order)

What is Kinetic Order?

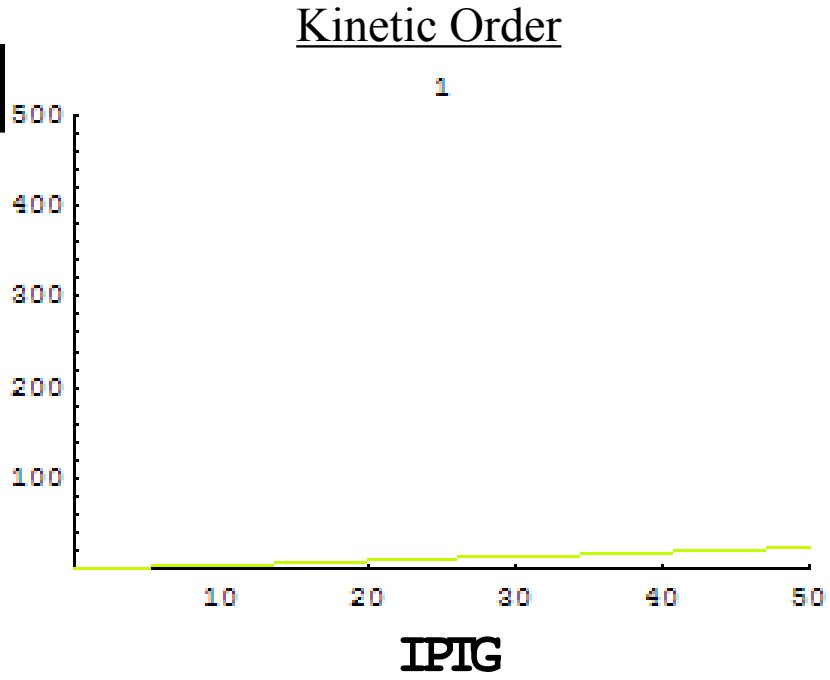
Consider:

$$\frac{d[RNA]}{d[IPTG]}$$

Hill coefficient

$$\frac{d[RNA]}{d[IPTG]} = k_1 \frac{IPTG^n}{k_2 + IPTG^n}$$

(Hill coefficient describes kinetic order)



Why Increase Kinetic Order?

- Increase Switch-like Behavior
- Increase Oscillations

Why Increase Kinetic Order?

- Increase Switch-like Behavior
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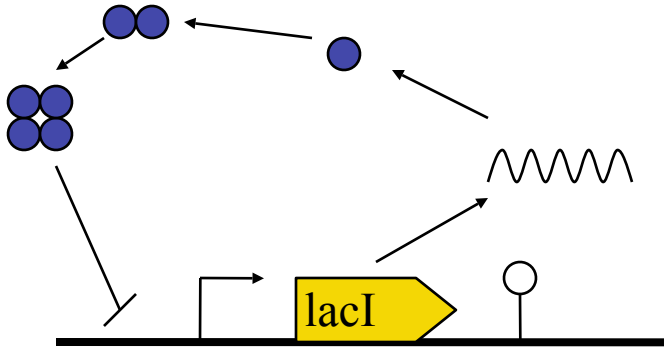
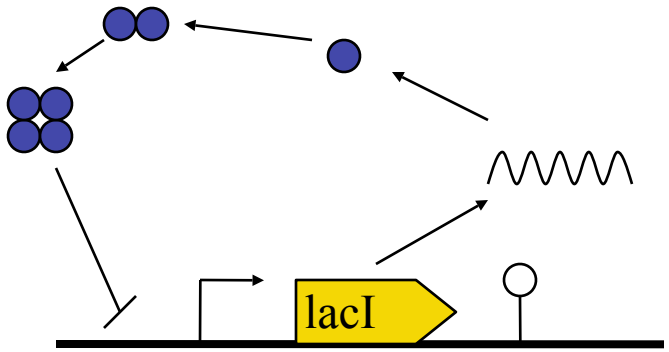
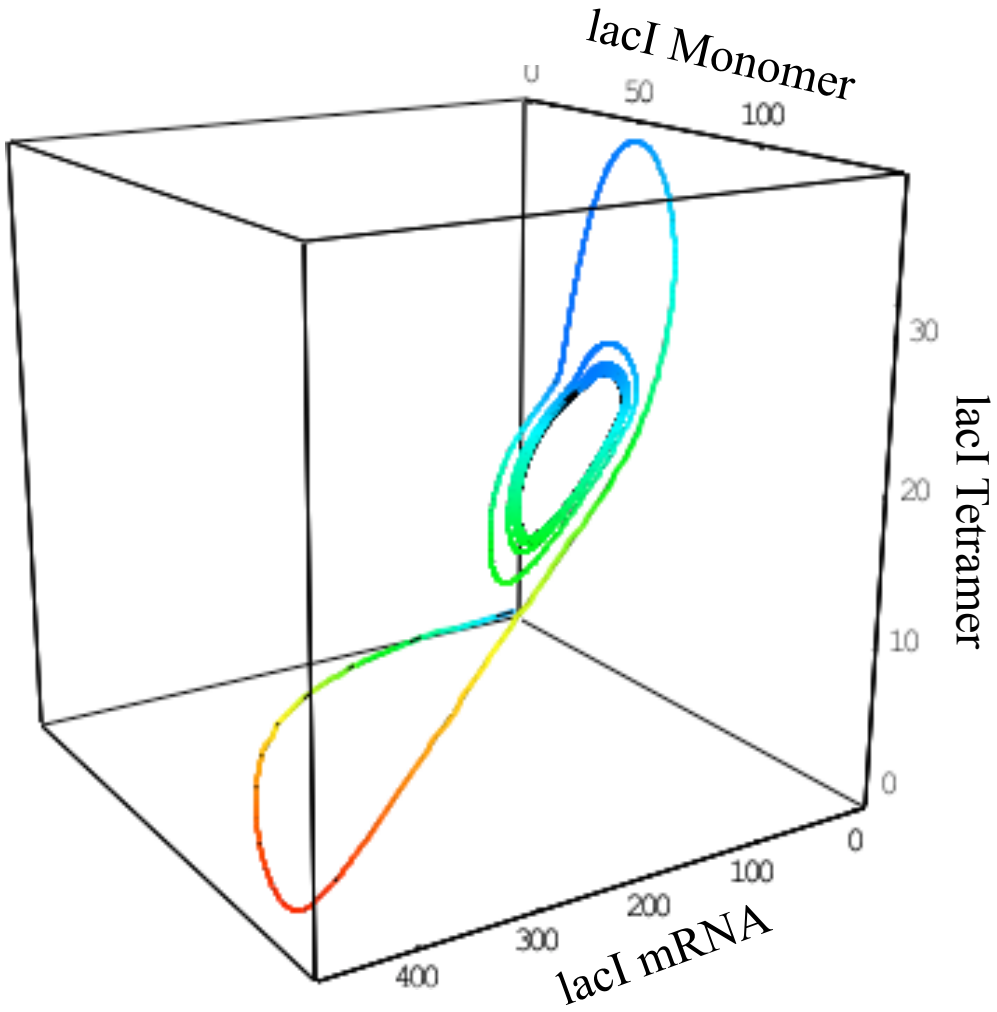


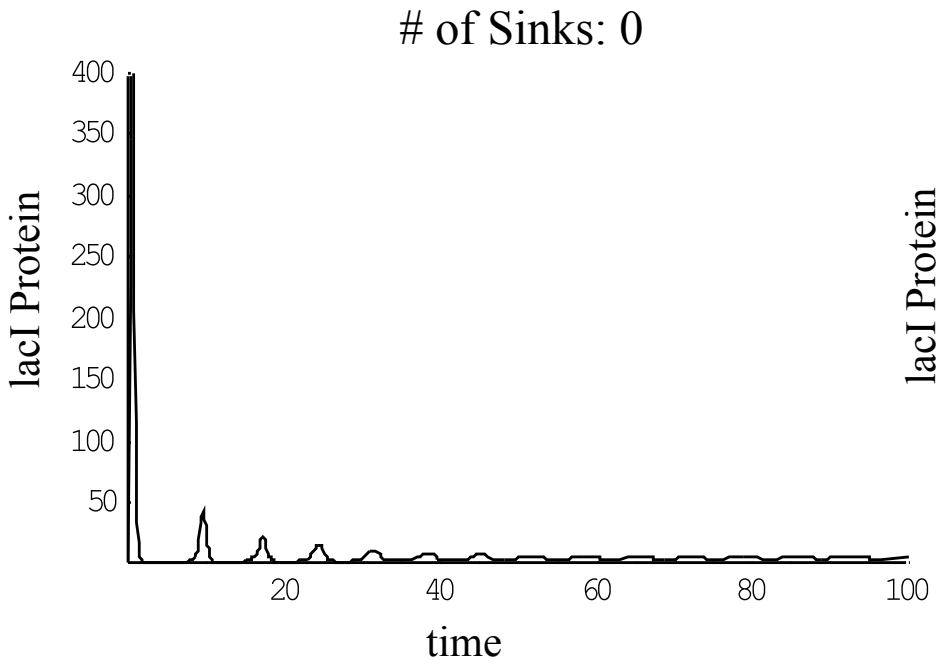
Why Increase Kinetic Order?

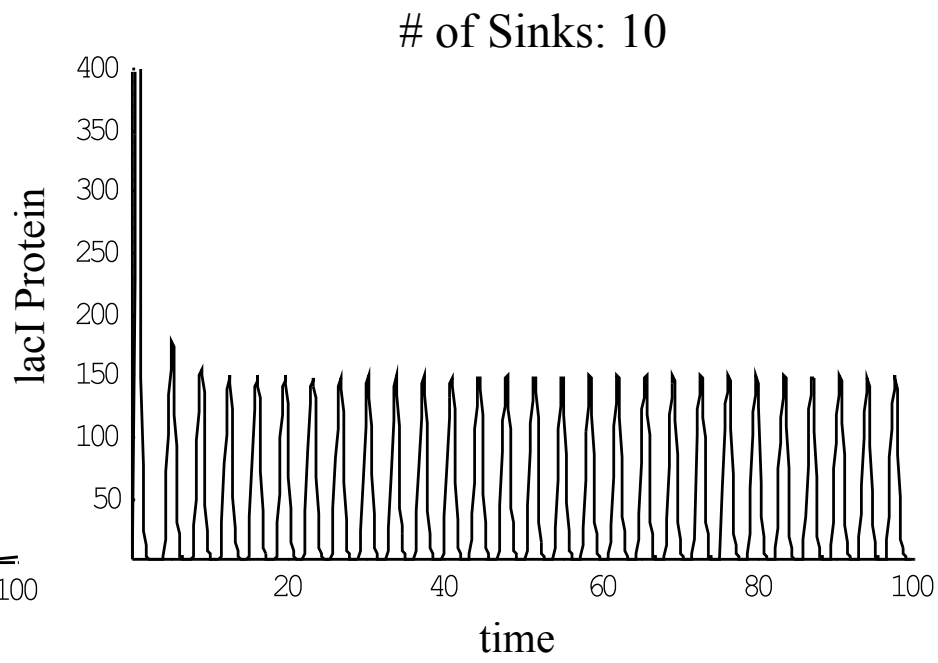
- Increase Switch-like Behavior
- Increase Oscillations





lacI Oscillator: Sinks vs. No Sinks





The Model

Definitions

Sc = Chromosomal sink,
Ss = Sink sites,
Sz = LacZYA promoter,
IP = IPTG,
m = Number of sink sites,
M = Number of chrosomal sink sites,
RSc = Repressor bound to non-specific binding site
RSs = Repressor bound to sink site,
RSz = Repressor bound to promoter
RIP = Repressor bound to IPTG H inactivated L
Rf = Free Repressor,
Rt = Total Repressor H constant L
R = Active Repressor H all repressor in system not bound to IPTG L
kc = k_{eq} for Non-specific binding site,
ks = k_{eq} for sink site,
kz = k_{eq} for promoter

Assumptions

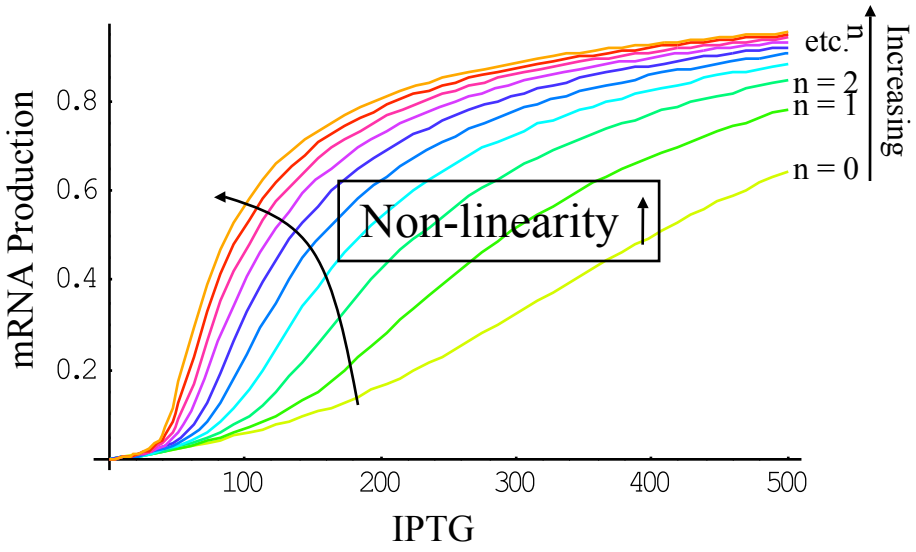
® Constant amount of total repressor,
® Quasi-equilibria H Repressor-IPTG, Repressor-Sink, Repressor-promoter L,
® ks = kz,
® kc is very small H 10^-6 L, so Rf << 1, kc* M ~ 1, so $\frac{kc\,M}{1+kc\,Rf}$ ~ 1

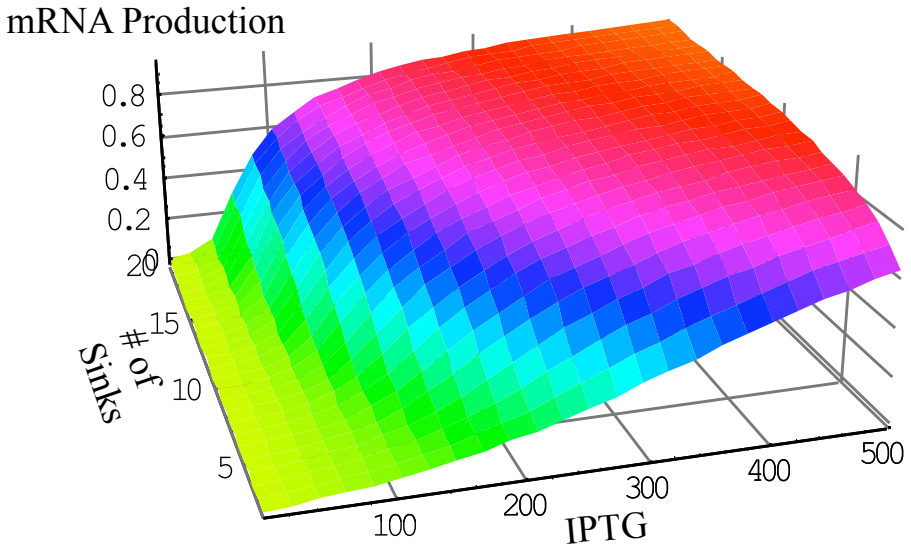
The Model

Rt Š Rf IP kip
M Š kc Rf
m Š ks
1 Š + kz Rf

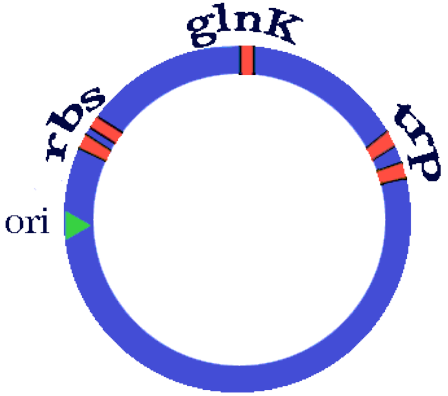
$\frac{Rt}{1+IP\,kip}$ Š Rf H 1 + $\frac{kc\,M}{1+kc\,Rf}$ + $\frac{ks\,m}{1+ks\,Rf}$ + $\frac{kz}{1+kz\,Rf}$ L

Increase in Kinetic Order with Sinks



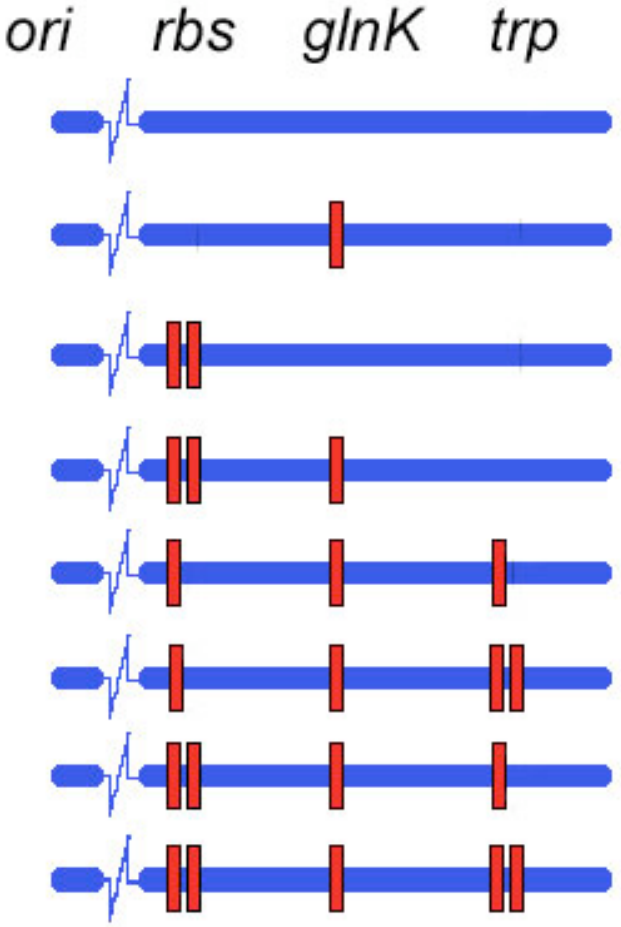


Experimental Manipulation of Lac Operon Kinetic Order

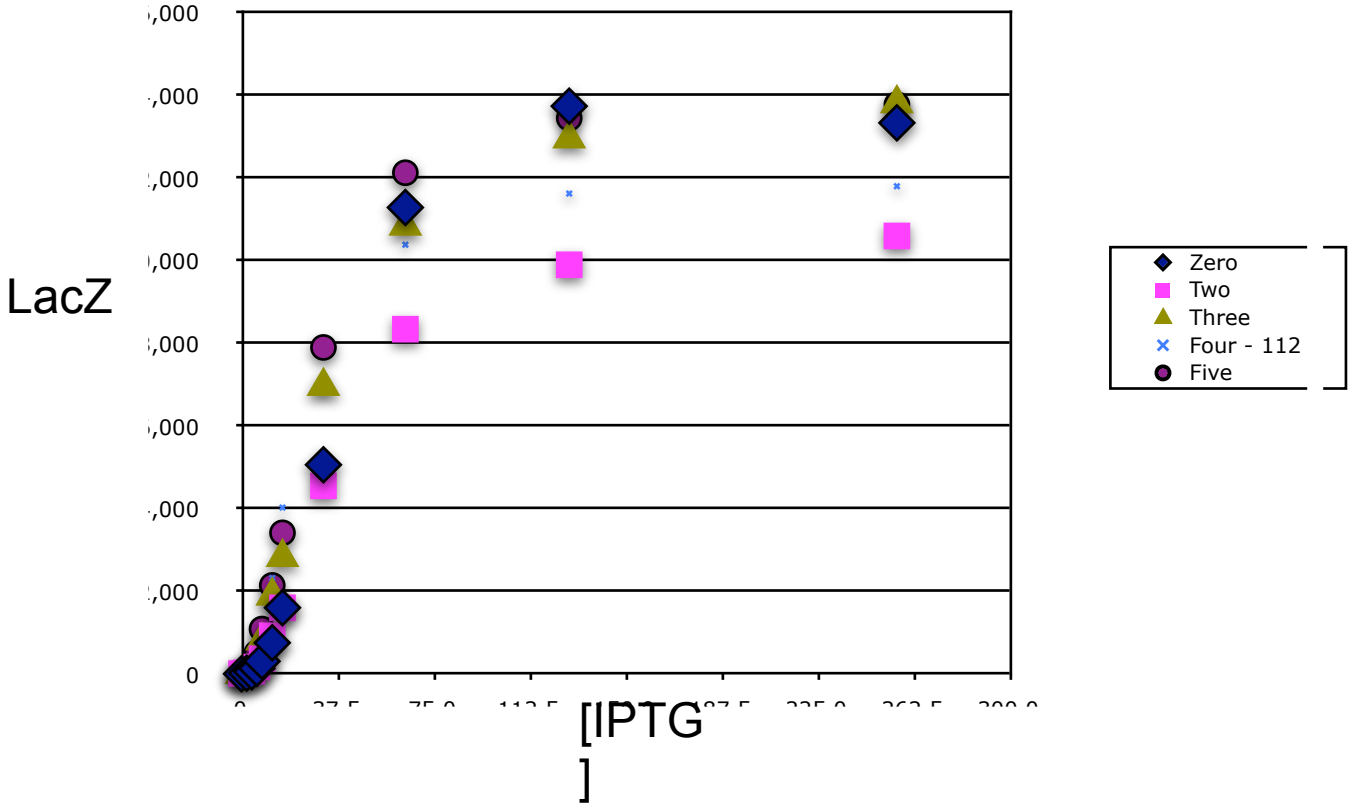


Sinks on Chromosome

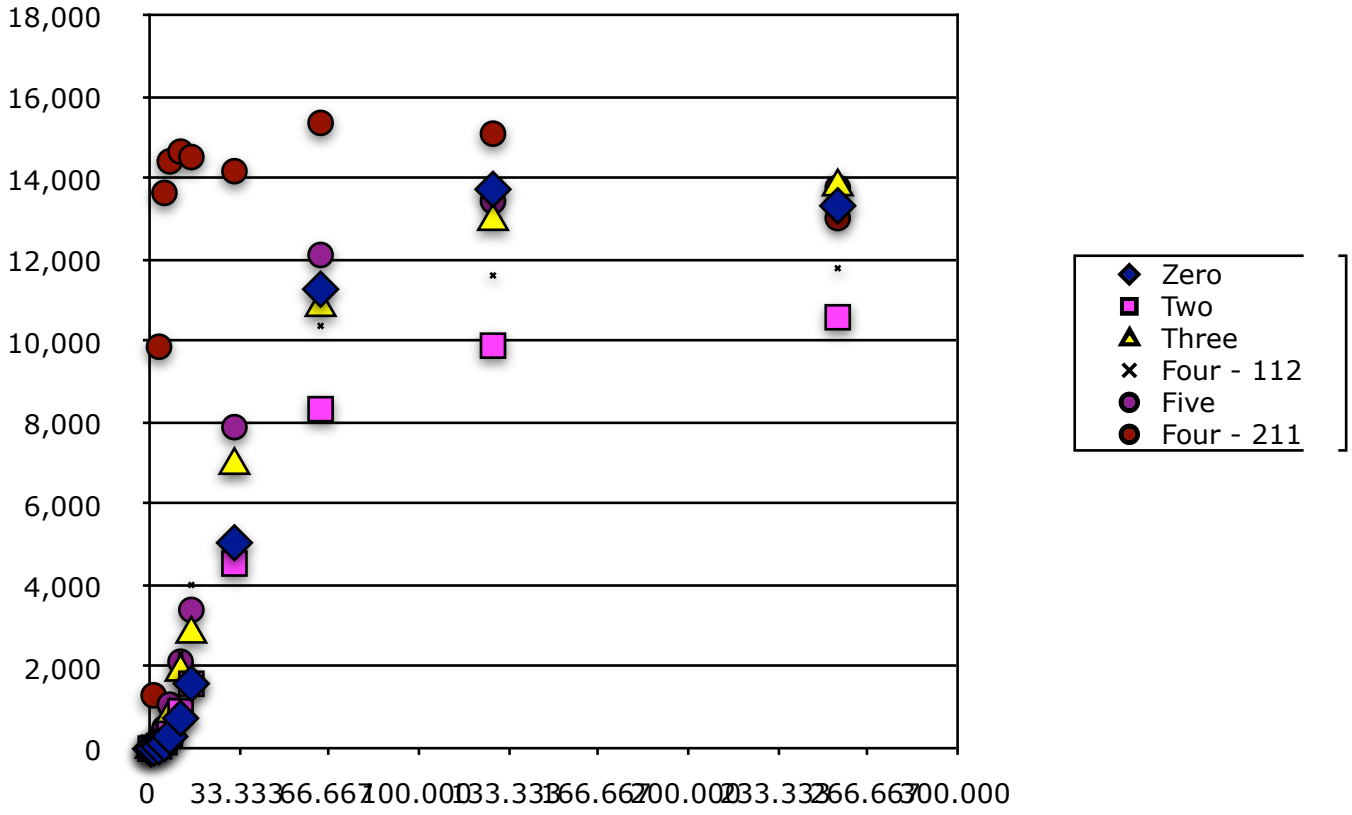
- O_p (red bars) inserted into chromosome using standard recombination methods
  - Landing pads
  - P1 vir transduction



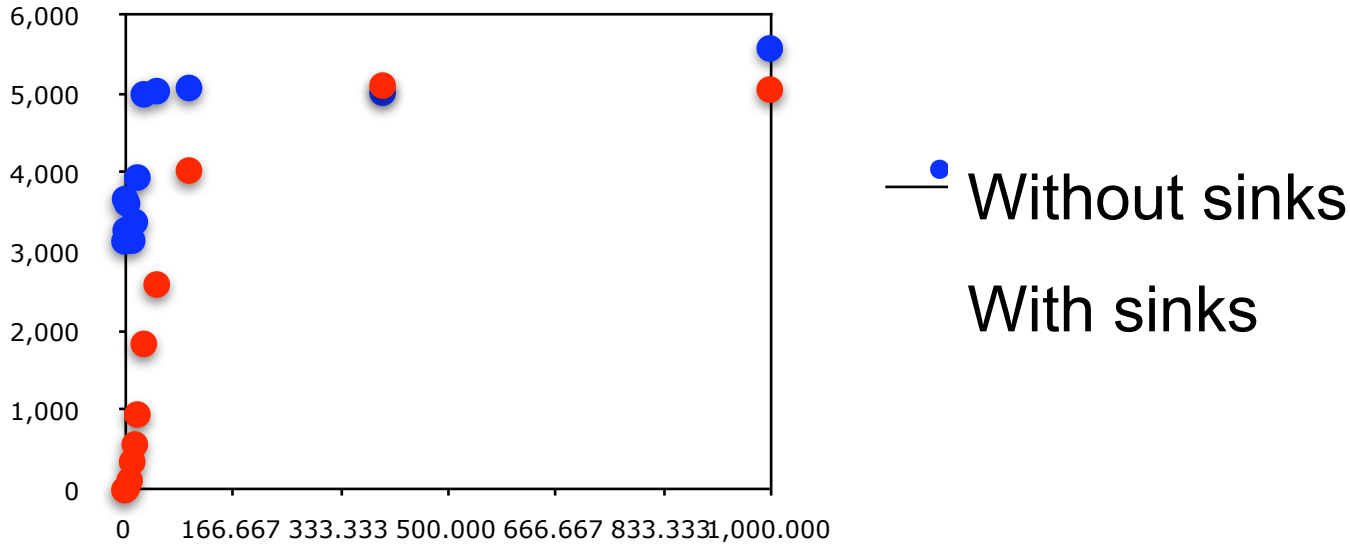
Increasing Number of Sinks on Chromosome Raises



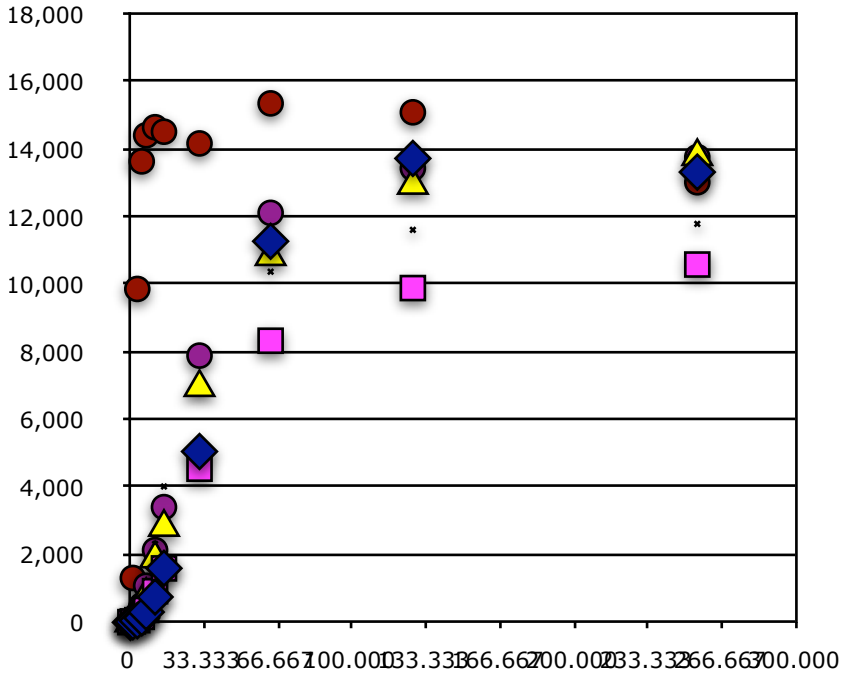
Strain with 4 Sinks Shows Switch-like Behavior

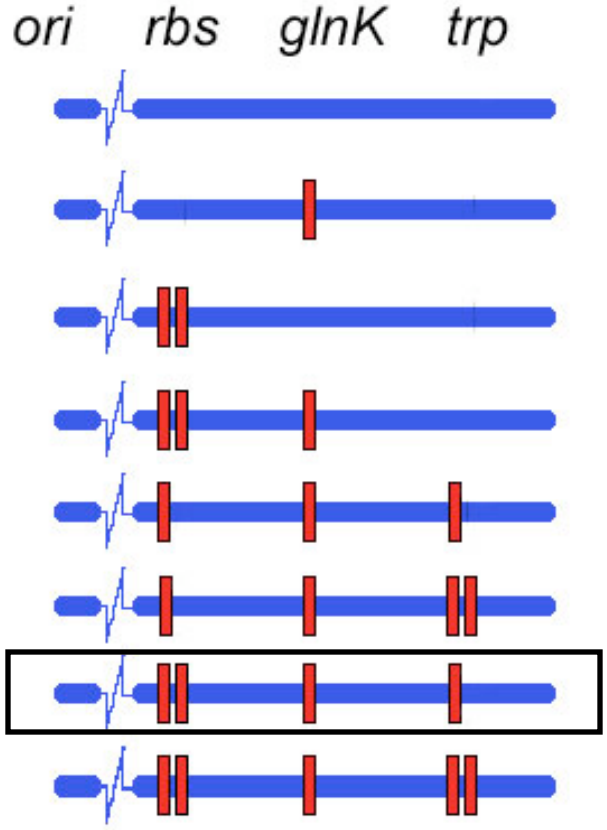


Sinks on Plasmid Raise Basal Level of LacZ Expression



Strain with 4 Sinks Shows Switch-like Behavior





Discussion

- Ongoing Work
  - Rebuild strains in different ways to see if we can repeat results
  - Examine effects of position and proximity
  - Examine effects of more weakly binding operators (O_1, O_2, etc.)
- Adding sinks to chromosome may allow for “tuning” of kinetic order

Overall Conclusion

- Work presented here was accomplished in a team effort
- Thanks go out to the many people who helped on the team's projects
- Go Blue!

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