

Engineering Genetic Circuits

Chris J. Myers

Lecture 2: Genetic Parts

Richard Feynman



What I cannot create,
I do not understand.

Know how to solve every
problem that has been solved

TO LEARN:
Bethe Ansatz Probs.
Kondo
2-D Hall,
order Temp
Non Linear Chemical Hydro

Why count \times sort

① $f = u(r, a)$
 $g = \frac{1}{2}(f, z) u(r, z)$
② $f = 21 f \cdot a / (u, a)$

- Richard Feynman, February 1988 -

Caltech Archives www.archives.caltech.edu

Genetic Parts

- Pieces of DNA that encode a biological function.
- Many correspond to well known sequence based genetic features.
 - Promoters
 - Ribosome binding sites
 - Protein coding sequences
 - Terminators
- The *Sequence Ontology* defines over 2800 types of sequence features.
<http://www.sequenceontology.org>
- Parts can be combined to produce devices (see next lecture).

Synthetic Biology Open Language (SBOL) Visual

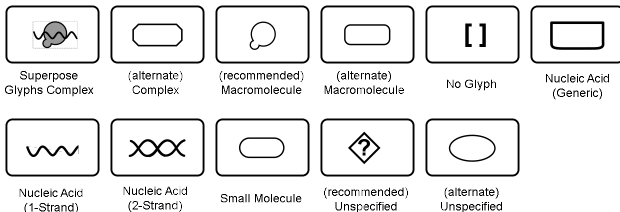
Nucleic Acid Glyphs

Aptamer	Assembly Scar	Blunt Restriction Site	(recommended) CDS	(alternate) CDS	Composite	Engineered Region	3' Overhang Sticky End
5' Overhang Sticky End	3' Sticky Restriction Site	5' Sticky Restriction Site	Insulator	No Glyph	Non-Coding RNA	Omitted Detail	Operator
ORI	ORI-T	Poly-A Site	Primer Binding Site	Promoter	Ribosome Entry Site	Signature	Recombination Site
Terminator	(recommended) Unspecified	(alternate) Unspecified	(recommended) DNA Location	(recommended) RNA Location	(recommended) Protein Location	(alternate) DNA Location	(alternate) RNA Location
(alternate) Protein Location	DNA Cleavage Site	RNA Cleavage Site	Protein Cleavage Site	DNA Stability Element	RNA Stability Element	Protein Stability Element	

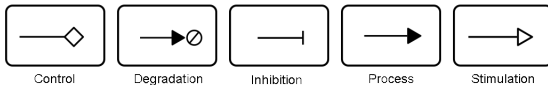
More information can be found at: <http://sbolstandard.org/visual/>

Synthetic Biology Open Language (SBOL) Visual

Molecular Species Glyphs

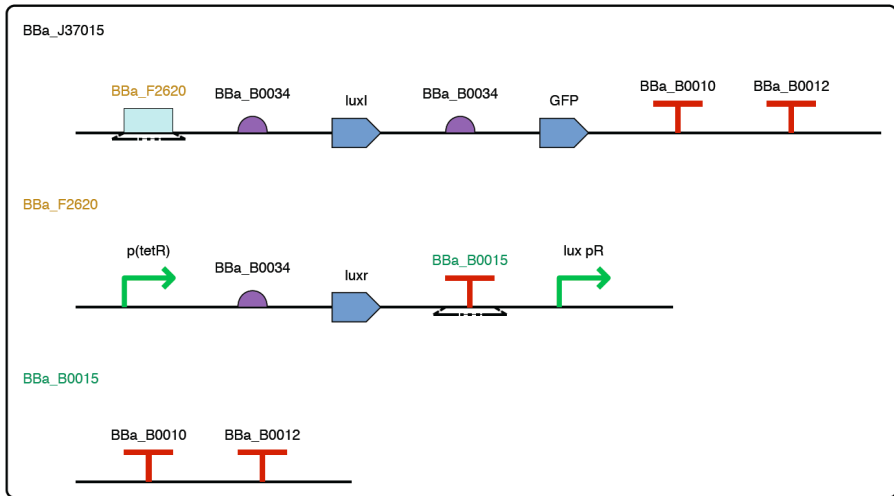


Interaction Glyphs



More information can be found at: <http://sbolstandard.org/visual/>

SBOL Visual Example



Rendered by VisBol (<http://visbol.org>)

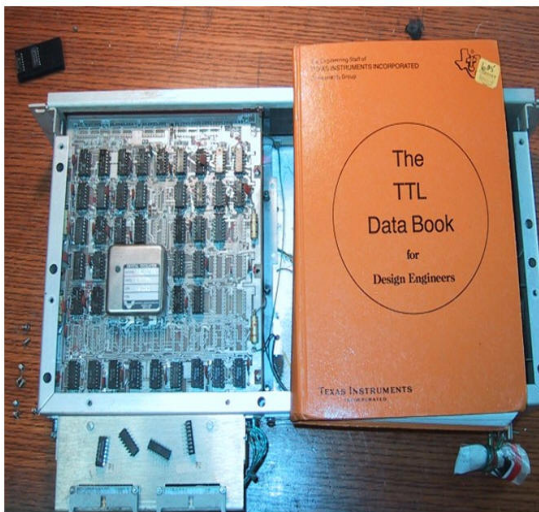
Standard Parts



Standard Parts




Standard Parts




<http://ee.hawaii.edu/~sasaki/EE260/Labs/Datasheets/7400.pdf>

iGEM Registry of Standard Biological Parts (BioBricks)

Registry of Standard Biological Parts

 tools catalog repository assembly protocols help search



Adding Parts to the Registry

The Registry's Repository contains thousands of documented parts with available DNA samples. Last year, iGEM teams submitted samples for over 2000 parts.

Be sure to add your parts and send samples to the Registry so that they can be made available to the community!

[Add a Part Sample Submission](#)

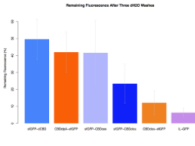
Featured Part

Cellulose Collection

Group: Team Imperial 2014, and others

The 2014 Imperial iGEM team created a bacterial cellulose filter for their **Aqualose** project. They wanted to produce flexible, and pollution-specific filters to aid in water sanitation. They created a set of well-documented cellulose binding domains, paired with reporter genes (GFP) and metal binding domains.

Many other teams have also worked with cellulose, so check out the cellulose related parts collection.



Part	Fluorescence Intensity
BBa-1030	~22
BBa-1031	~18
BBa-1032	~18
BBa-1033	~12
BBa-1034	~8
L-GFP	~2

Catalog

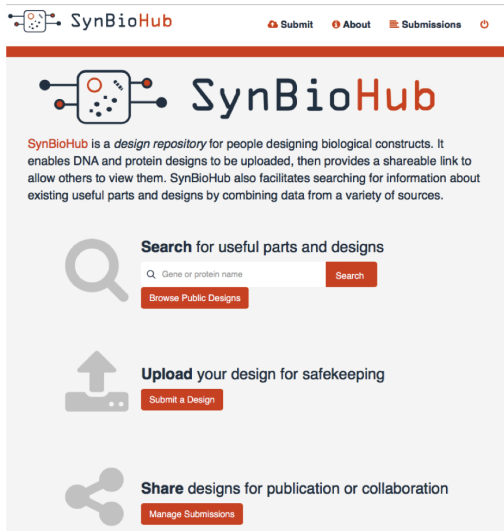
The iGEM Registry has over 20,000 documented parts. The Catalog organizes many of these parts by part type, chassis, function, and more. Browse for parts through the Registry Catalog or use the search menu.

2017 DNA Distribution

The iGEM 2017 DNA Distribution has started shipping! We've added some new material this year, so be sure to read through the 2017 Distribution Handbook for storage instructions and how to use your kit!

<http://parts.igem.org>

SynBioHub Data Repository



James McLaughlin
Anil Wipat



Zach Zundel
Chris Myers

Version 1.0 released
June 14, 2017

Reference Instance (<https://synbiohub.org>)



SynBioHub

Submit

About

Submissions

Admin



[Advanced Search](#) | [Create Collection](#) | [SPARQL](#)

Bacillus subtilis Collection

version 1

This collection includes information about promoters, operators, CDSs and proteins from *Bacillus subtilis*. Functional interactions such as transcriptional activation and repression, protein production and various protein-protein interactions are also included.



IGEM Parts Registry

version 1

The iGEM Registry is a growing collection of genetic parts that can be mixed and matched to build synthetic biology devices and systems. As part of the synthetic biology community's efforts to make biology easier to engineer, it provides a source of genetic parts to iGEM teams and academic labs.



IGEM 2017 Distribution

version 1

Distribution of parts for the 2017 iGEM competition



SBOL Compliant Software

version 1

A collection of software that supports the Synthetic Biology Open Language (SBOL) standard



ACS Synthetic Biology

version current



BU/MIT Living Computing Project

(<https://synbiohub.programmingbiology.org>)

The screenshot displays the SynBioHub interface. At the top, there is a navigation bar with links for 'Submit', 'About', 'Submissions', and 'Admin'. Below this is a search bar with the placeholder text 'Search SynBioHub' and a green 'Search' button. A link for 'Advanced Search | Create Collection | SPARQL' is also visible. The main content area features a list of collections, each with a title, version, description, and a logo. The collections are: 'Cello Parts' (version 1, public), 'Living Computing Project' (version 1, public), 'BU BDC Lab ICE' (version current, private), 'MIT SBC Lab ICE' (version current, private), and 'Cidar Lab Benchling' (version current, private). Each collection's logo is displayed to the right of its description.

Cello Parts
version 1
A collection of parts used in the Cello Science Paper.

Living Computing Project
version 1
Designs created as part of the NSF Expeditions Living Computing Project

BU BDC Lab ICE
version current
BU Biological Design Center Lab's Inventory

MIT SBC Lab ICE
version current
MIT Synthetic Biology Center Lab's Inventory

Cidar Lab Benchling
version current
Cidar Lab Benchling collection of parts



COMPUTATIONAL
BIOSCIENCE RESEARCH
CENTER

SBOLme

repository of SBOL parts for metabolic engineering

Query Catalog Information About

SBOLme is a repository of [SBOL 2.0-compliant](#) metabolic parts for metabolic engineering. Here, you can search for these biological parts from the SBOLme repository with convenient criteria for composing biosynthesis systems.

Search for

Compound

Filter By

Compound name or ID

Value

Pyruvate, C00022, ME_C00022

Search

25

With this query, you can search for a compound by its name or its IDs. The acceptable IDs are the KEGG compound IDs and the SBOLme compound IDs.

Page 1 of 1 results

SBOLme ID	KEGG ID	Name
ME_R00006	R00006	2-acetolactate pyruvate-lyase (carboxylating)

Kuwahara et al., ACS Synthetic Biology (2017)

Includes 28,437 chemical compounds, 6,883 enzyme classes,
9,909 metabolic reactions, and 3,173,238 proteins from 3,908 organisms.

ICE (<https://acs-registry.jbei.org>)

ACS SyntheticBiology

Search SEARCH

myers@ece.utah.edu

Create Entry

COLLECTIONS

- Featured 287
 - Linshiz et al. 2012 6
 - Hillson et al. 2012 27
 - Hagen et al. 2015 34
 - Sania et al. 2014 54
 - Phelan et al. 2014 15
 - Shih et al. 2015 64
 - Linshiz et al. 2014 40
- Personal 35
- Shared 12
- Drafts 0

WEB OF REGISTRIES

- > JBEI Registry
- > JBEI Public Registry
- > MIT Synthetic Biology

FEATURED Hillson et al. 2012 Public 1/25/16 7:58 PM

TYPE	PART ID	NAME	STATUS	CREATED
PART	ACS_000032	DclpX_cassette Linear cassette for markerless genomic deletion of clpX gene from E...	Complete	Aug 9, 2011
STRAIN	ACS_000031	JBEI-3083 clpX markerless deletion in Keasling-1484 background	Complete	Aug 9, 2011
STRAIN	ACS_000030	JBEI-2948 Strain for linear arabinose response transformed with pREDi. Interme...	Complete	Aug 9, 2011
STRAIN	ACS_000029	JBEI-3140 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000028	JBEI-3139 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000027	JBEI-3138 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000026	JBEI-3137 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000025	JBEI-3136 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000024	JBEI-3135 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000023	JBEI-3134 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000022	JBEI-3133 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011
STRAIN	ACS_000021	JBEI-3144 clpX protease markerless deletion in Keasling-1484 background, for L...	Complete	Aug 9, 2011

DOI Bioenergy Research Centers

UC DAVIS

CARNEGIE INSTITUTION FOR SCIENCE

Lawrence Livermore National Laboratory

Pacific Northwest National Laboratory

JBEI ICE Registry 1.1.1
All rights reserved.
Submit an Issue | Help

Ham et al., Nucleic Acid Research (2012)

Other Useful Repositories

- **NCBI** - <https://www.ncbi.nlm.nih.gov>
 - **PubMed** - <https://www.ncbi.nlm.nih.gov/pubmed>
 - **GeneBank** - <https://www.ncbi.nlm.nih.gov/genbank>
 - **Protein** - <https://www.ncbi.nlm.nih.gov/protein>
 - **Many others** - <https://www.ncbi.nlm.nih.gov/guide/all/>
- **EMBL-EBI** - <http://www.ebi.ac.uk>
 - **BioModels** - <http://www.ebi.ac.uk/biomodels-main/>
 - **ChEBI** - <http://www.ebi.ac.uk/chebi/>
 - **Reactome** - <http://reactome.org>
 - **UniProt** - <http://www.uniprot.org>
 - **Many others** - <http://www.ebi.ac.uk/services/all>
- **addgene** - <https://www.addgene.org>
- **Pathway Commons** - <http://www.pathwaycommons.org>

Data Standards: FASTA

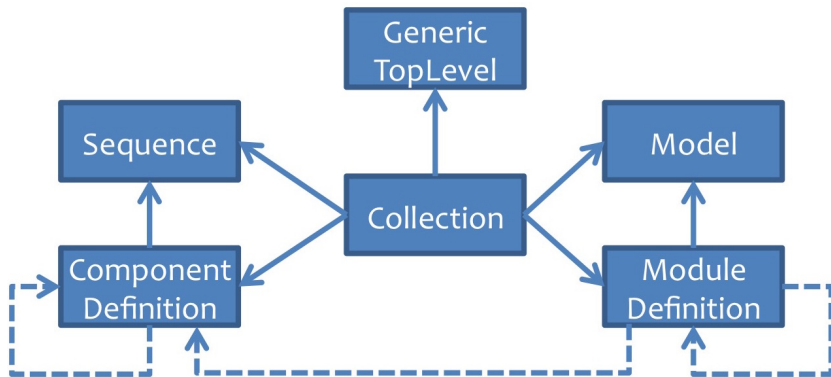
```
>BBa_B0015 sequence 1 (129 bp)
ccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcggttttatctgt
tgtttgtcgggtgaacgctctctactagagtcacactggctcaccttcgggtgggccttt
ctgcgtttata
```

Data Standards: GenBank

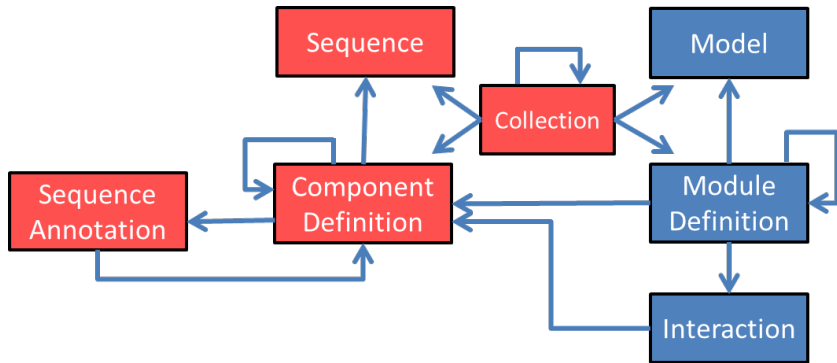
```
LOCUS      BBa_B0015              129 bp    DNA      linear    UNK 23-Aug-20
DEFINITION double terminator (B0010-B0012)
ACCESSION  BBa_B0015
VERSION    BBa_B0015.1
FEATURES             Location/Qualifiers
     misc_feature     12..55
     misc_feature     1..80
     terminator       1..80
     misc_feature     96..115
     misc_feature     122..122
     polyA_site       116..129
     misc_feature     89..129
     terminator       89..129
ORIGIN
      1 ccaggcatca aataaaacga aaggctcagt cgaaagactg ggcctttcgt tttatctgtt
     61 gtttgtcggg gaacgctctc tactagagtc aactggctc accttcgggt gggcctttct
    121 gcgtttata
//
```

- Provides support for:
 - Incomplete sequences.
 - Hierarchical designs.
 - Non-DNA components.
 - Functional interactions.
 - External models.
 - Arbitrary annotations.
- Serialized in XML/RDF format.
- Leverages *semantic web* database technology.
- There are several library implementations of the SBOL data structure, which provide an *application programmers interface* (API) for tool developers to interact with SBOL data objects.

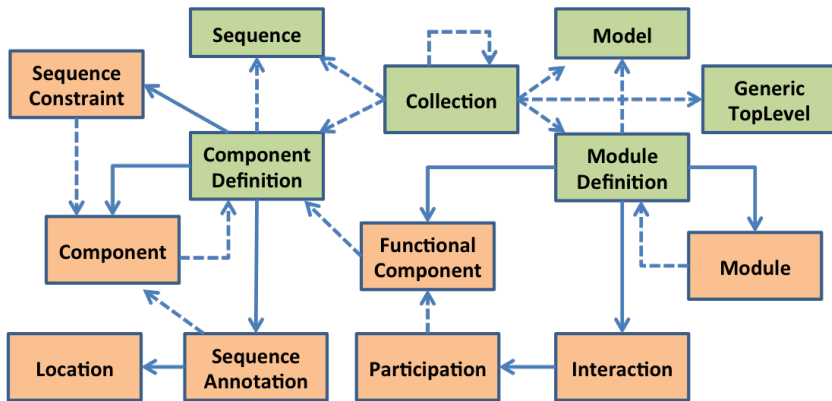
Data Standards: SBOL



Data Standards: SBOL



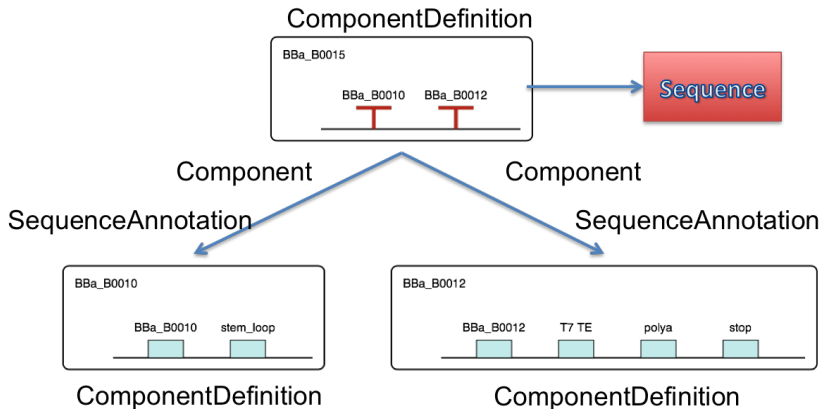
Data Standards: SBOL



SBOL 2.2 Updates

- Attachment class - serves as a general container for data files.
- Implementation class - represents a physical instance of a synthetic biological construct.
- CombinatorialDerivation class - specifies combinatorial genetic designs without having to specify every possible design variant.
- Provenance ontology (Prov-O):
 - Activity class - something that occurs over a period of time and acts upon or with entities.
 - Agent class - something that bears some form of responsibility for an activity taking place.
 - Plan class - an entity that represents a set of actions or steps intended by one or more agents to achieve some goals.

SBOL Example



Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL



Part:BBa_F2620:Design

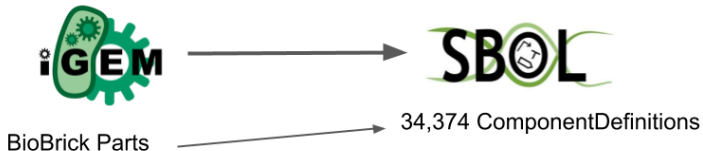
Designed by: Barry Canton [bcanton@mit.edu] and An

3OC₆HSL → PoPS Receiver



ComponentDefinition

Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL



34,374 ComponentDefinitions

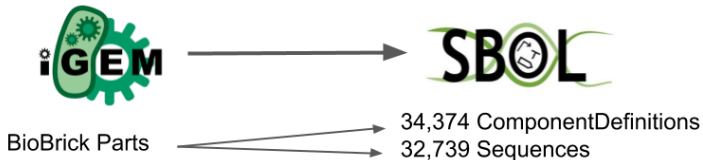
>BBa_F2620 Part-only sequence (1061 bp)

```
tcoccatcagtgatagagattgacatccctatcagtgatagagatactgagcactactagagaaagaggagaaatactagatgaaaaacataaatgccga  
cgacacatacagaataaattaataaaaattaaagcttctagaagcaataatgatattaatcaatgcttatctgatatgactaaaatgggtacattgtgaatat  
tattttactcgcgatcattttatcctcattctatggttaaatctgatatatttcaatcctagataaattacocctaaaaaatggaggcaatattatgatgacgcta  
atttaataaaaatgatcctatagtagattattctaaactccaatcattccaccaattaatgggaatatatttgaataaatgctgtaataaaaaatctcc  
aatgtaattaaagaagcgaaaaacatcaggtcttatcactgggttttagtttccctattcctacggctaacaatggcttcggaatgcttagtttgcacat  
tcagaaaaagacaactataatagatagtttattttacatgcgtgtatgaacataccattaattgttctctctagttgataattatcgaaaaataaata  
tagcaataataaatcaaacacagatttaaccaaaagagaaaaagaatgtttagcgtgggcatgcgaaggaagaaagctcttgggatatttcaaaaatatt  
agggtgcagtgagcgtactgtcactttccatttaaccaatgcgcaaatgaaactcaatacaacaacccgctgccaaagtatttctaagcaattttaaca  
ggagcaattgattgcccactactttaaaaatataaacactgatagtgtctagtgtagatcactactagagccaggcatcaataaaacgaaaggctcagtc  
gaaagactggggccttctgtttatctgtttgttgcgtgaacgctctctactagagtcacactggctcaccctcgggtgggcctttctgcgtttatata  
ctagagacctgtaggatcgtacaggtttacgcaagaaaatgggtttgtatagtcgaataaa
```



Sequence

Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL



Part:BBa_F2620:Design

Designed by: Barry Canton [bcanton@mit.edu] and An

34,374 ComponentDefinitions

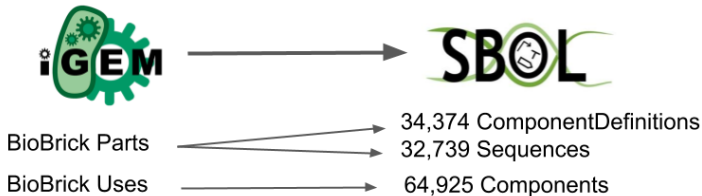
32,739 Sequences

3OC₆HSL -> PoPS Receiver



Components

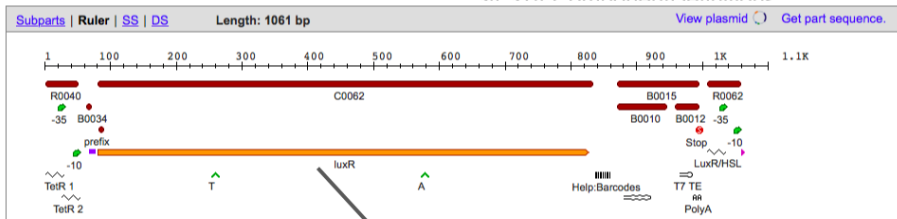
Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL

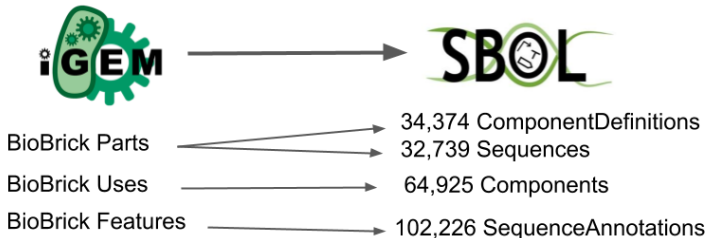


34 374 ComponentDefinitions



SequenceAnnotations

Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL



iGEM Part/Feature Type	SequenceOntology (SO) Term
Coding	CDS (SO:0000316)
E Device	Engineered Region (SO:0000804)
E Primer	Primer (SO:0000112)
E Protein Domain	Polypeptide Domain (SO:0000417)
RBS	Ribosome Entry Site (SO:0000139)
Regulatory	Promoter (SO:0000167)
Tag	Tag (SO:0000324)
Terminator	Terminator (SO:0000141)
etc.	etc.

Conversion of the iGEM Registry to SBOL



BioBrick Parts

34,374 ComponentDefinitions

32,739 Sequences

BioBrick Uses

64,925 Components

BioBrick Features

102,226 SequenceAnnotations

27 Part / 20 Feature Types

Sequence Ontology (SO)
(includes 2817 terms)


Conversion of the iGEM Registry to SBOL



Page Header

;

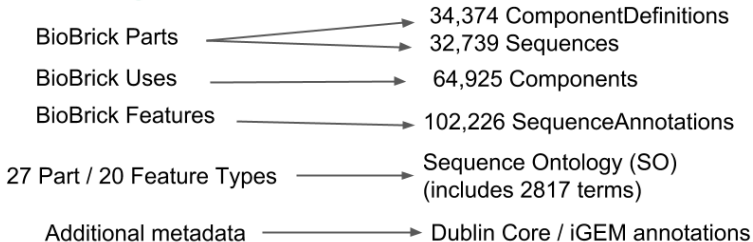
Login to edit

Part Name	BBa_F2620
Short Description	3OC ₆ HSL -> PoPS Receiver
Part Type	Signalling 
Nickname	
Designer(s)	Barry Canton [bcanton@mit.edu] and Anna Labno [labnoa@mit.edu]
DNA Status	Available
Qualitative Experience	Works
Group Favorite	No
Star Rating	1
Delete This Part	Not Deleted

S

Dublin Core/iGEM Annotations

Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL



BioBrick Parts

34,374 ComponentDefinitions

32,739 Sequences

BioBrick Uses

64,925 Components

BioBrick Features

102,226 SequenceAnnotations

27 Part / 20 Feature Types

Sequence Ontology (SO)
(includes 2817 terms)

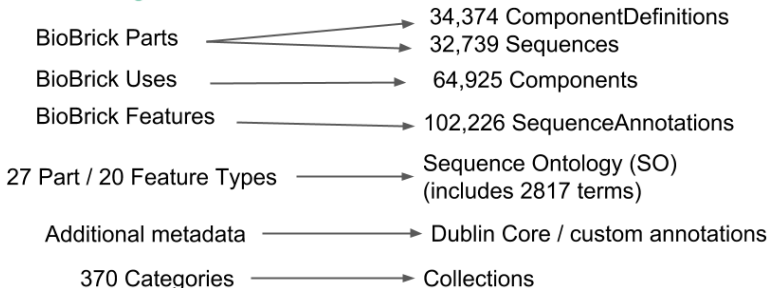
Categories

//classic/signalling/receiver
//function/cellsignalling

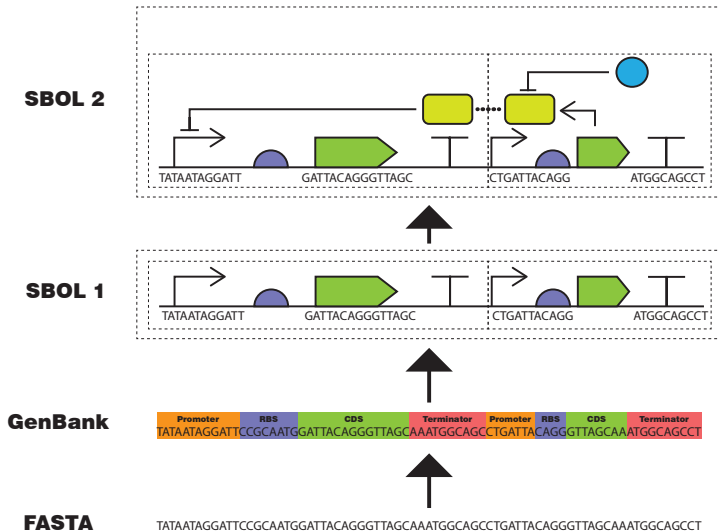
Dublin Core / custom annotations

Collections

Conversion of the iGEM Registry to SBOL



Comparison of Standard Data Formats



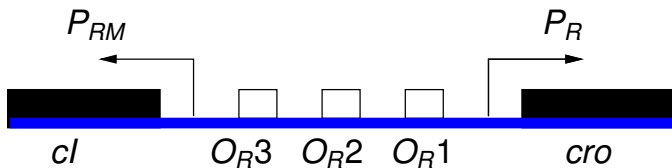
Promoters

- Recruit transcriptional machinery.
 - RNA polymerase
 - Other accessory proteins
- Lead to transcription of the downstream DNA sequence producing RNA.
- Can be thought of as a part that produces a certain number of RNA molecules per unit time.
- Constitutive
 - Not regulated
 - Always on
- Regulated
 - Activity is modulated by the presence of other protein factors.
 - Positive regulation
 - Negative regulation
- Strength of promoters varies - sequence and spacing of -10/-35 matters
- See <http://parts.igem.org/Promoters>

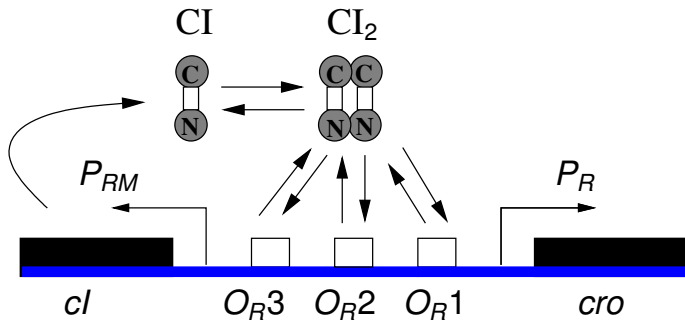
Operators

- Sequences that recruit the binding of *transcription factors* that regulate promoter activity.
- Classically operators recruit repressors to negatively regulate promoters.
- Also some operators bind activators (positive regulators).
- Often not considered as separate parts - included within the promoter.
- Strength of regulation mediated by the sequence of the operator.
- Can be manipulated to tune the level of repression or activation.

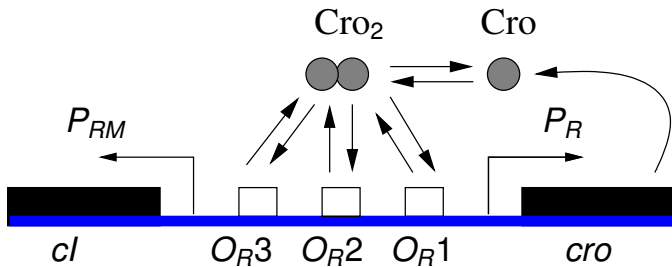
The O_R Operator



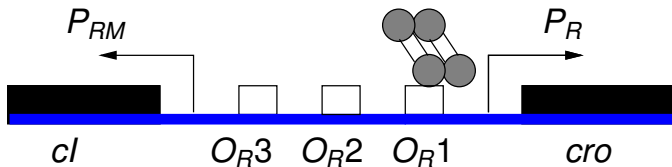
CI, the λ Repressor



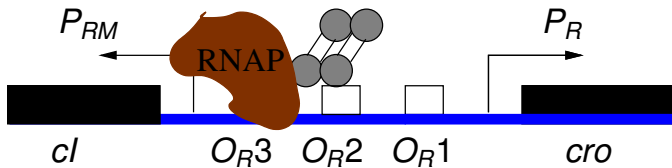
λ 's Cro Molecule



Cl_2 Bound to O_{R1} Turns Off P_R



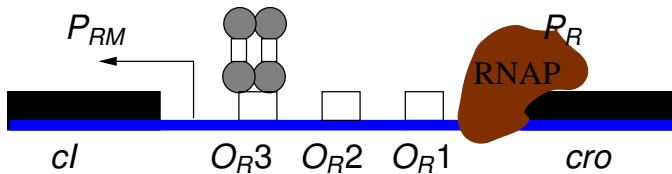
Cl_2 Bound to O_{R2} Turns On P_{RM}



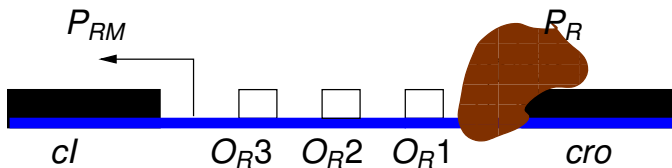
Basal Versus Activated Rate

- Nothing in biology is clear-cut.
- Without Cl_2 bound to O_{R2} , RNAP can still bind to P_{RM} and initiate transcription of Cl at a reduced *basal rate*.
- With Cl_2 bound to O_{R2} , transcription occurs at enhanced *activated rate*.

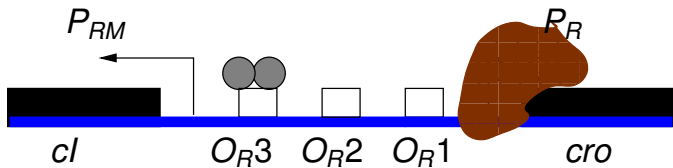
Cl_2 Bound to O_{R3} Turns Off P_{RM}



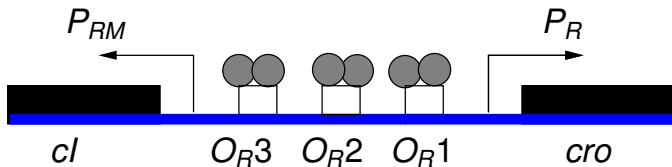
P_R Active When O_R Sites Are Empty



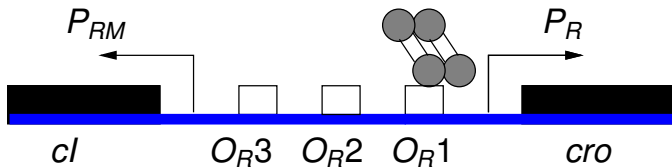
Cro_2 Bound to O_{R3} Turns off P_{RM}



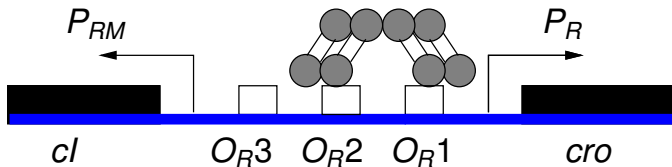
Cro_2 Bound to O_{R1} or O_{R2} Turns off P_R



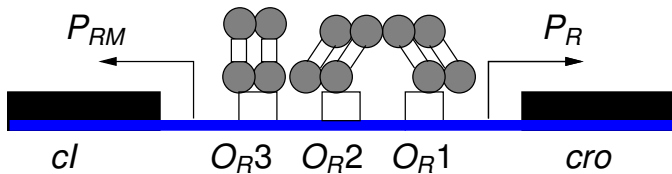
Low Concentrations of Cl_2



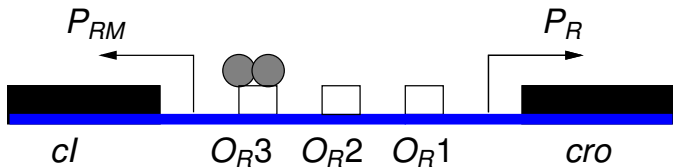
Cooperativity Aids Cl_2 Binding to O_{R2}



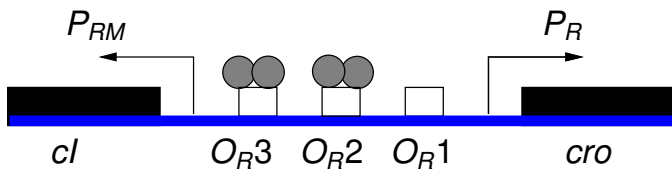
High Concentrations of Cl_2



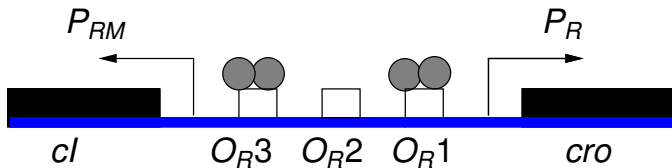
Low Concentrations of Cro₂



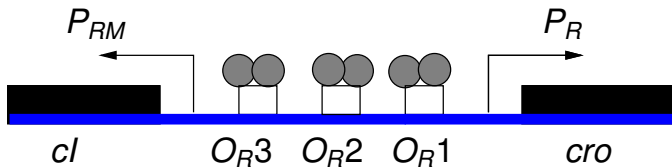
Moderate Concentrations of Cro₂



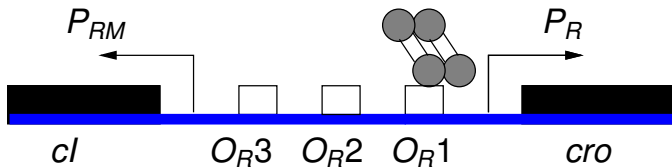
OR



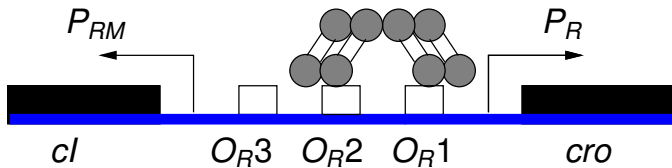
High Concentrations of Cro₂



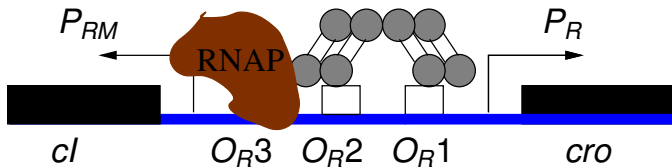
Cooperativity of Cl_2 Binding



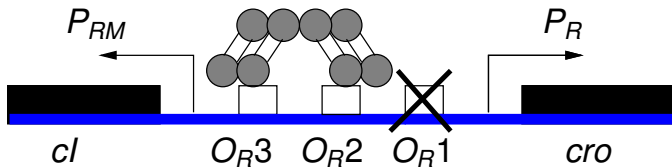
Cooperativity of Cl_2 Binding



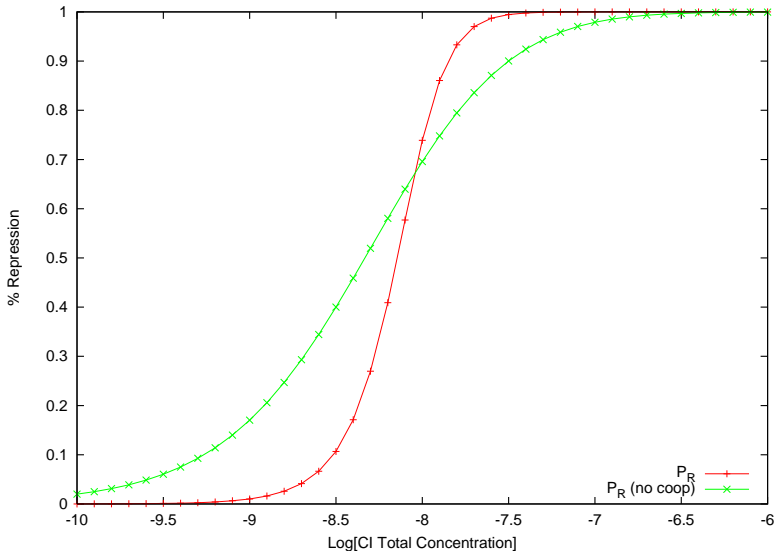
Cooperativity of Cl_2 Binding



Cooperativity of Cl₂ Binding



Effect of Cooperativity



Configurations of O_R

- At low to moderate concentrations of Cl and Cro , there are three common configurations:
 - No molecules bound to O_R , Cro produced at full rate and Cl produced at low basal rate.
 - Cl_2 bound to O_R1 and O_R2 , Cro production repressed, and Cl activated.
 - Cro_2 bound to O_R3 , Cl cannot be produced, Cro is produced.
- Feedback of the products as transcription factors coupled with affinities makes O_R behave as a bistable switch.
- In *lysis* state, Cro produced locking out production of Cl .
- In *lysogeny* state, Cl produced locking out production of Cro .

Recognition of the Operators

- Cl_2 and Cro_2 bind to operator sites that are 17 base pairs long.
- How do these proteins locate these sequences from amongst the millions within the bacteria?
- Observing from midpoint, a strand on one side is nearly symmetric with complimentary strand on other side.

Near Symmetry in the Operator Sequences

<i>Op</i>	Operator sequences	Operator half sequences
O_{R1}	$T A T C A C C G c C A G A G G T A$ $A T A G T G G C g G T C T C C A T$	$T A T C A C C G c$ $T A C C T C T G$
O_{R2}	$T A A C A C C G t G C G T G T T G$ $A T T G T G G C a C G C A C A A C$	$T A A C A C C G t$ $C A A C A C G C$
O_{R3}	$T A T C A C C G c A A G G G A T A$ $A T A G T G G C g T T C C C T A T$	$T A T C A C C G c$ $T A T C C C T T$
O_{L1}	$T A T C A C C G c C A G T G G T A$ $A T A G T G G C g G T C A C C A T$	$T A T C A C C G c$ $T A C C A C T G$
O_{L2}	$T A T C T C T G g C G G T G T T G$ $A T A G A G A C c G C C A C A A C$	$T A T C T C T G$ $C A A C A C C G c$
O_{L3}	$T A T C A C C G c A G A T G G T T$ $A T A G T G G C g T C T A C C A A$	$T A T C A C C G c$ $A A C C A T C T$
<i>Con.</i>		$T_9 A_{12} T_6 C_{12} A_9 C_{11} C_7 G_9 C_5$ $C_2 C_3 T_2 T_1 T_4 T_2 T_1$ $A_1 A_3 C_1 G_1 C_1$

Consensus Sequence

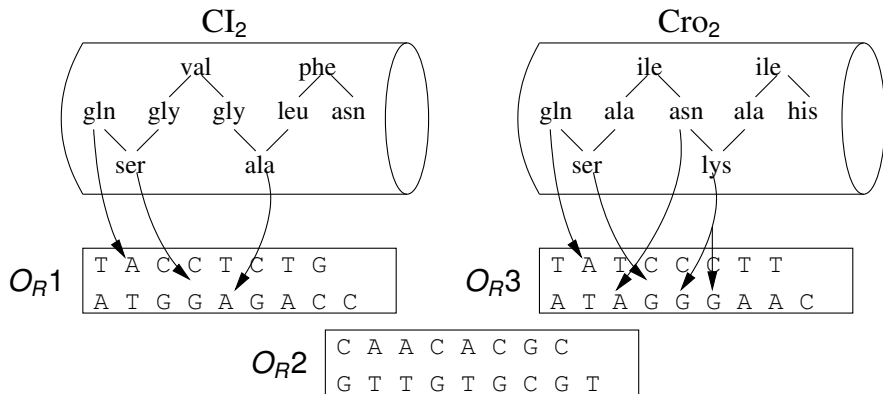
- The *consensus sequence* is as follows:

TATCACCGcCGGTGATA

ATAGTGGCgGCCACTAT

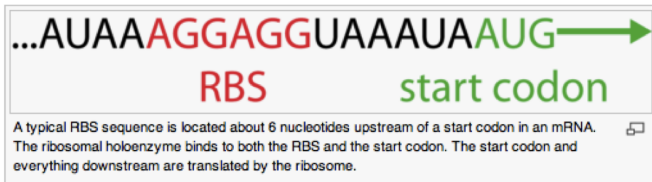
- Many entries are highly preserved.
- Differences exist that cause the differences in affinity for Cl_2 and Cro_2 for the different operators.
- Notice that the first half of the operator sites O_R1 and O_R3 agree perfectly with the consensus sequence while second half has several differences.

Amino Acid-Base Pair Interactions



Ribosome Binding Sites

- A sequence in mRNA to which ribosomes bind and initiate translation.
- Bacteria usually require RBS and start codon.
- Also called *Shine-Dalgarno sequence*.
- See http://parts.igem.org/Ribosome_Binding_Sites

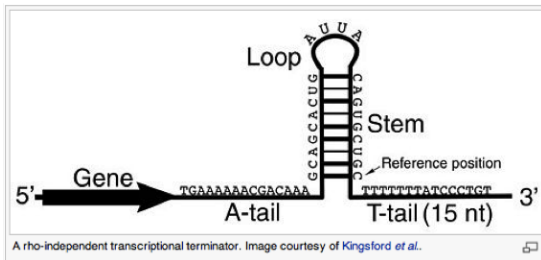


Protein Coding Regions

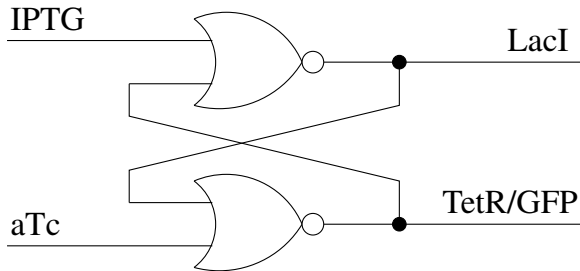
- Specify an amino acid sequence for a protein using the genetic code.
- Begin with a start codon, typically ATG to initiate translation.
- End with a stop codon, TAA, TAG, TGA to end translation.
- See http://parts.igem.org/Protein_coding_sequences

Terminators

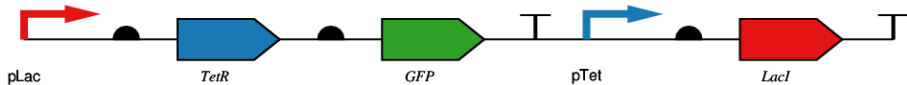
- Sequence of DNA that signals the end of transcription.
- Placed past 3' end of the protein coding sequence.
- Often palindromic in sequence in prokaryotes.
- Weakens interaction between RNAP and the DNA strand being copied.
- Two categories in prokaryotes:
 - Rho dependent
 - Rho independent
- Rho independent most commonly used.
- See <http://parts.igem.org/Terminators>



Genetic Toggle Switch (SR Latch) Logic Diagram



Genetic Toggle Switch (Gardner et al. 2000)



Assignment #1

- ➊ Ensure that you understand genetic circuits to the level of detail presented in lecture 1.
- ➋ Read the genetic toggle switch paper:
<http://www.nature.com/nature/journal/v403/n6767/full/403339a0.html>
- ➌ Read the paper that you selected for this course.
- ➍ Download and examine the supplemental material for your paper.
- ➎ Create an account on <https://synbiohub.utah.edu>.
- ➏ Locate parts needed to construct a genetic toggle switch.
 - ➊ Submit the parts into a collection in your private repository.
 - ➋ Explain your choices in the details fields.
 - ➌ Provide a share link to your private repository.
- ➐ Locate parts needed to construct your paper's genetic circuit.
 - ➊ Submit the parts into a collection in your private repository.
 - ➋ Explain your choices in the details fields.
 - ➌ Provide a share link to your private repository.