

A Synthetic Biology Workflow Using SBOL

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SEED 2017
June 23, 2017

Reproducibility in Synthetic Biology

Essential information for synthetic DNA sequences

To the Editor:

Following a discussion by the workgroup for Data Standards in Synthetic Biology, which met in June 2010 during the Second Workshop on Biodesign Automation in Anaheim, California, we wish to highlight a problem relating to the reproducibility of the synthetic biology literature. In particular, we have noted the very small number of articles reporting synthetic gene networks that disclose the complete sequence of all the constructs they describe.

To our knowledge, there are only a few examples where full sequences have been released. In 2005, a patent application¹ disclosed the sequences of the toggle switches published four years earlier in a paper by Gardner *et al.*². The same year, Basu *et al.*³ deposited their construct sequences for programmed pattern formation into GenBank³. Examples of synthetic DNA sequences derived from standardized parts that have been made available in GenBank include the refactored genome of the bacteriophage

gaps between key components are almost never reported, presumably because they are not considered crucial to the report. Yet, synthetic biology relies on the premise that synthetic DNA can be engineered with base-level precision.

Missing sequence information in papers hurts reproducibility, limits reuse of past work and incorrectly assumes that we know fully which sequence segments are important. For example, many synthetic biologists are currently realizing that translation initiation rates are dependent on more than the Shine-Dalgarno sequence⁴. Sequences upstream of the

start codon are crucial for translation rates, yet are underreported. Similarly, it has been demonstrated that intron length can affect the dynamics of genetic oscillators⁵. Many more such examples are likely to emerge.

Because full sequence disclosure is critical, we wonder why the common requirement by many journals to provide GenBank entries

for genomes and natural sequences has

and welcome contributions from the greater community.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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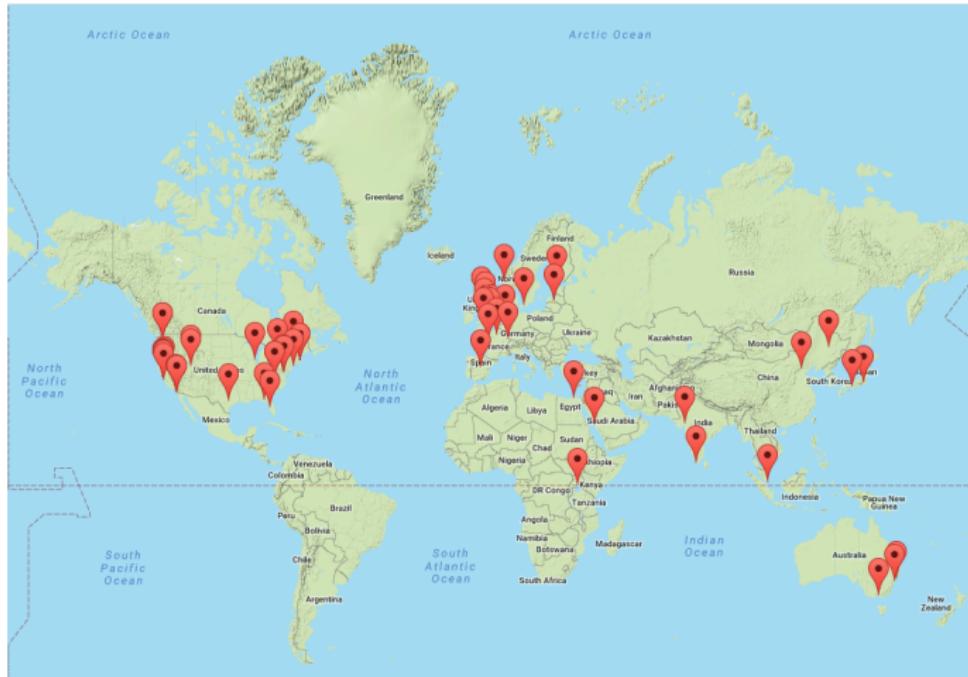
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1. Gardner, T.S. & Collins, J.J. US patent 6,841,376 (2005).
2. Gardner, T.S., Cantor, C.R. & Collins, J.J. *Nature* **403**, 339–342 (2000).
3. Basu, S., Gerchman, Y., Collins, C.H., Arnold, F.H. & Voigt, C.A. *Nature* **431**, 118–124 (2004).



Synthetic Biology Open Language (SBOL) Community



128 people from 16 countries

Representing 43 universities and 29 companies and government labs

Organizations Supporting SBOL



Current support for the development of SBOL provided by National Science Foundation Grants DBI-1356041 and DBI-1355909, and the Engineering and Physical Sciences Research Council under Grant Number EP/J02175X/1.

Synthetic Biology Open Language (SBOL)

Version 1 Released in 2011

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NATURE BIOTECHNOLOGY | COMPUTATIONAL BIOLOGY | PERSPECTIVE

日本語要約

The Synthetic Biology Open Language (SBOL) provides a community standard for communicating designs in synthetic biology

Michał Galdzicki, Kevin P Clancy, Ernst Oberortner, Matthew Pocock, Jacqueline Y Quinn,
Cesar A Rodriguez, Nicholas Roehner, Mandy L Wilson, Laura Adam, J Christopher Anderson,
Bryan A Bartley, Jacob Beal, Deepak Chandran, Joanna Chen, Douglas Densmore, Drew
Endy, Raik Grünberg, Jennifer Hallinan, Nathan J Hillson, Jeffrey D Johnson, Allan Kuchinsky,
Matthew Lux, Goksel Misirli, Jean Peccoud, Hector A Plahar, Evren Sirin, Guy-Bart Stan, Alan
Villalobos, Anil Wipat, John H Gennari, Chris J Myers & Herbert M Sauro

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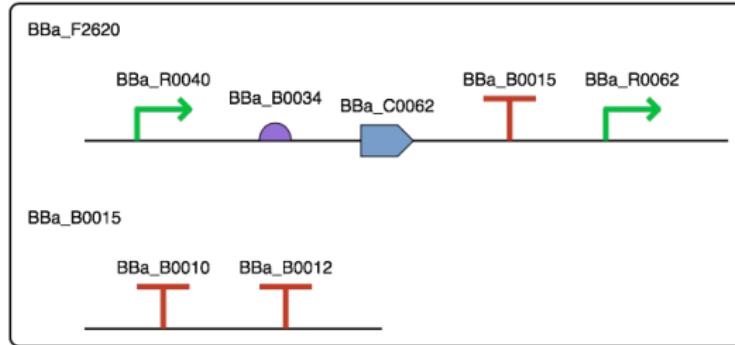
[Affiliations](#) | [Contributions](#) | [Corresponding author](#)

Nature Biotechnology 32, 545–550 (2014) | doi:10.1038/nbt.2891
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COMMUNITY PAGE

SBOL Visual: A Graphical Language for Genetic Designs

Jacqueline Y. Quinn^{1*}, Robert Sidney Cox III^{2*}, Aaron Adler³, Jacob Beal³, Swapnil Bhatia⁴, Yizhi Cai⁵, Joanna Chen^{6,7}, Kevin Clancy⁸, Michal Galdzicki⁹, Nathan J. Hillson^{6,7}, Nicolas Le Novère¹⁰, Akshay J. Maheshwari¹¹, James Alastair McLaughlin¹², Chris J. Myers¹³, Umesh P¹⁴, Matthew Pocock^{12,15}, Cesar Rodriguez¹⁶, Larisa Soldatova¹⁷, Guy-Bart V. Stan¹⁸, Neil Swainston¹⁹, Anil Wipat¹², Herbert M. Sauro^{20*}



SBOL Version 2 Released in 2015

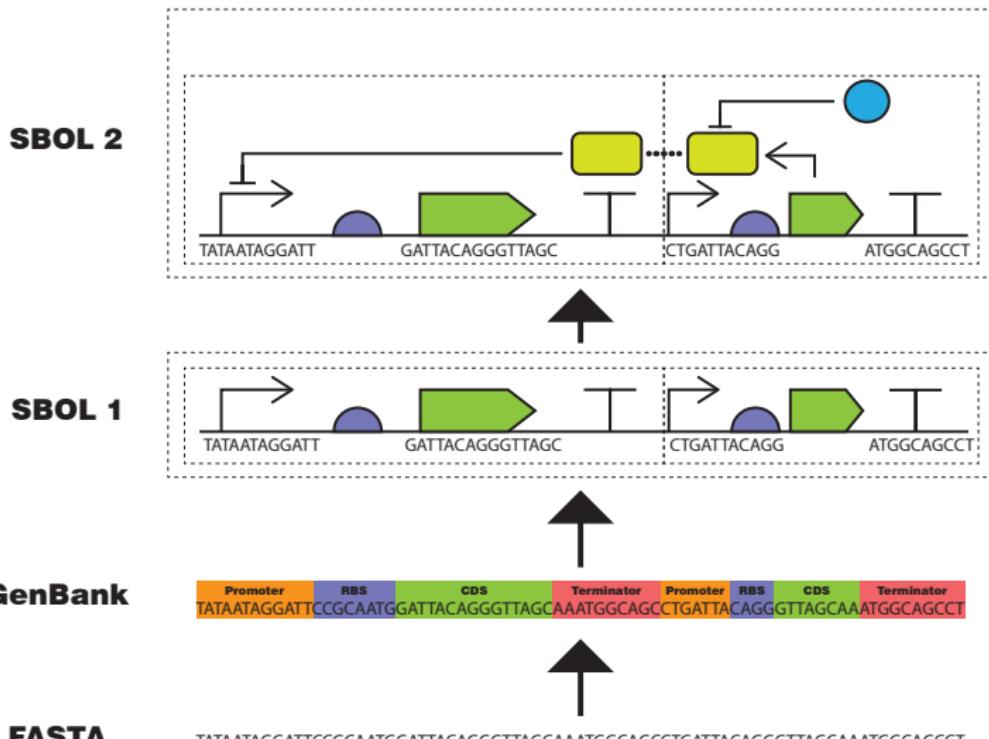
Sharing Structure and Function in Biological Design with SBOL 2.0

Nicholas Roehner,^{*,†} Jacob Beal,[‡] Kevin Clancy,[§] Bryan Bartley,[⊥] Goksel Misirli,^{||} Raik Grünberg,[¶] Ernst Oberortner,[#] Matthew Pocock,[▽] Michael Bissell,[⊗] Curtis Madsen,^{||} Tramy Nguyen,[■] Michael Zhang,[■] Zhen Zhang,[■] Zach Zundel,[▲] Douglas Densmore,[†] John H. Gennari,[●] Anil Wipat,^{||} Herbert M. Sauro,[⊥] and Chris J. Myers[■]

SEE ALSO:

- Roehner et al., ACS Synthetic Biology (2014)
- Bartley et al., Journal of Integrative Biology (2015)
- Beal et al., Journal of Integrative Biology (2016)

SBOL Evolution



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

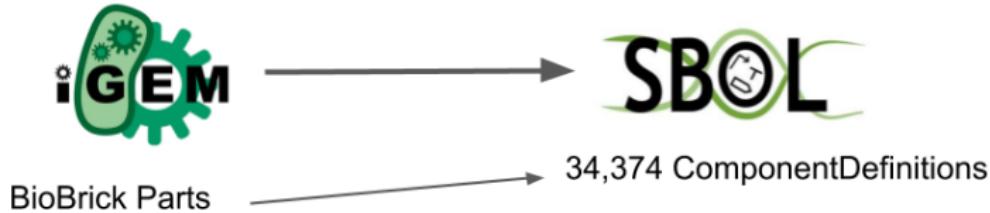
p(tetR) luxR lux pR
R0040 B0034 C0062 B0015 R0062



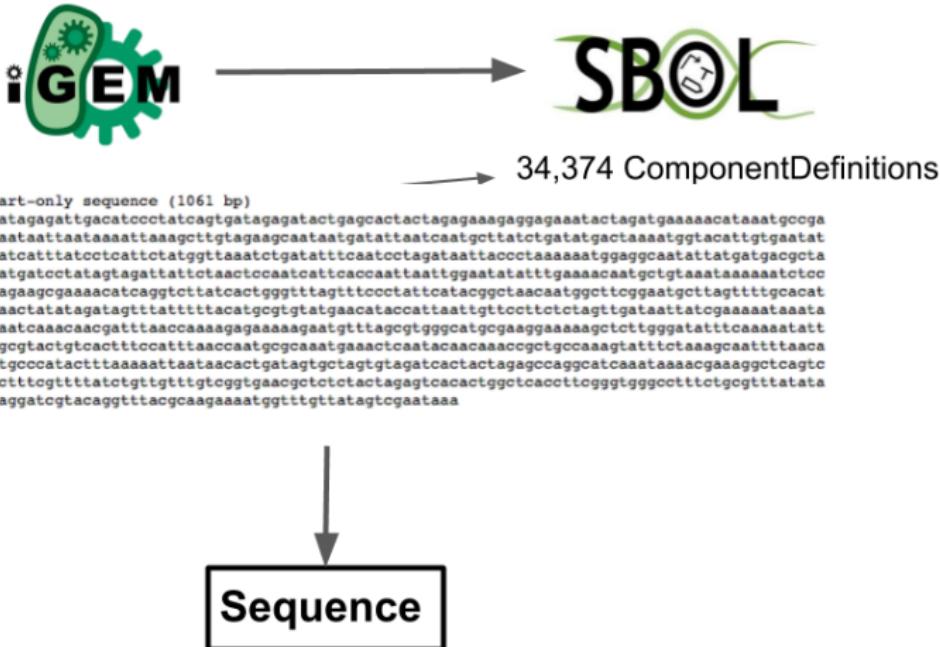
A schematic diagram of a genetic circuit. It starts with a promoter labeled 'p(tetR)' followed by a green arrow pointing to a green oval representing the TetR protein. This is followed by a purple arrow pointing to a red oval representing the 3OC₆HSL molecule. A second green arrow points from the red oval to a second green oval representing the PoPS protein, which is under the control of a promoter labeled 'lux pR'.

ComponentDefinition

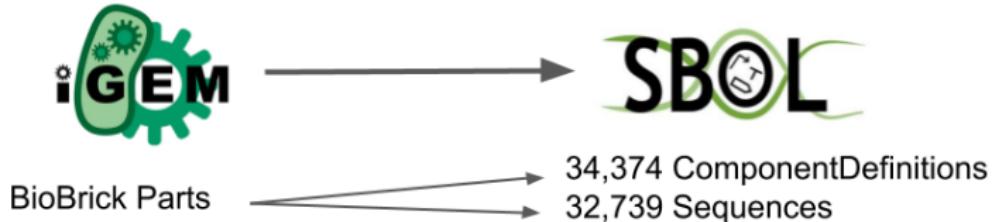
Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL



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

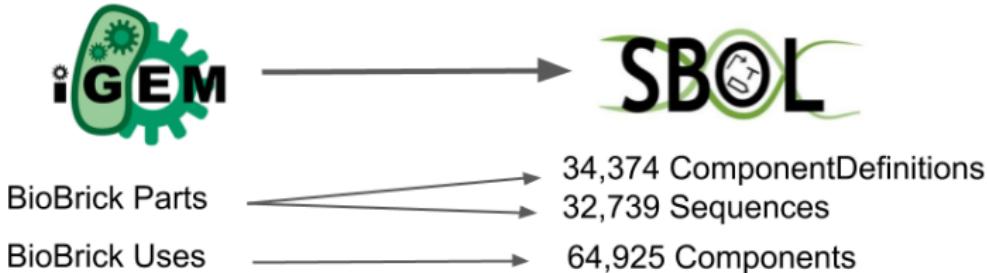
3OC₆HSL → PoPS Receiver

p(tetR)
R0040 B0034 luxr
C0062 B0015 lux pR
R0062



Components

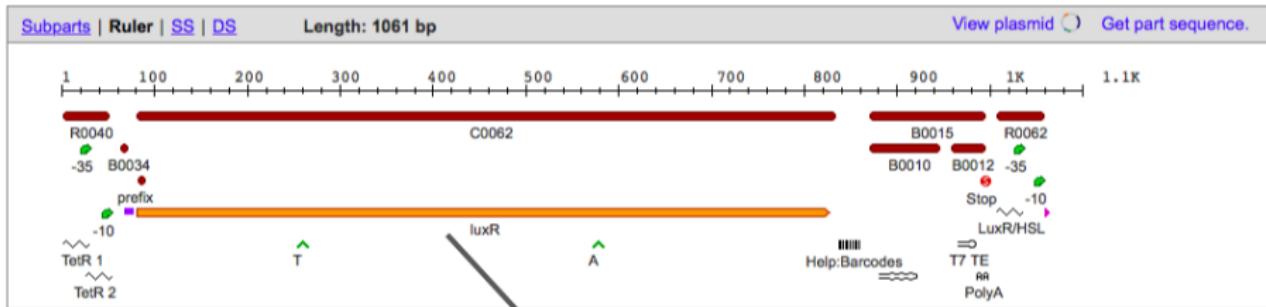
Conversion of the iGEM Registry to SBOL



Conversion of the iGEM Registry to SBOL

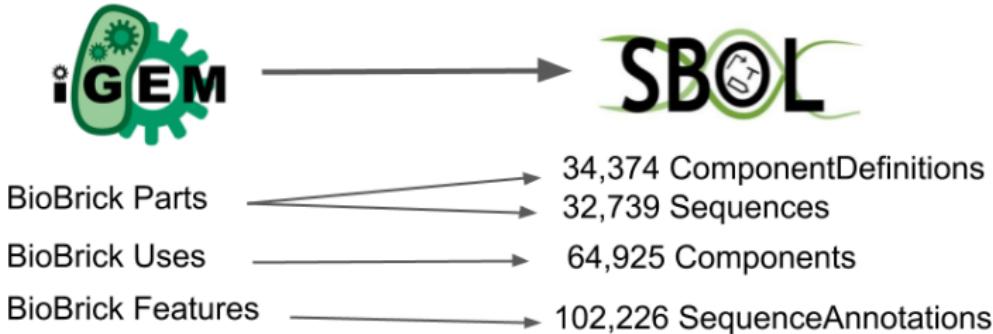


24,374 ComponentDefinitions



SequenceAnnotations

Conversion of the iGEM Registry to SBOL

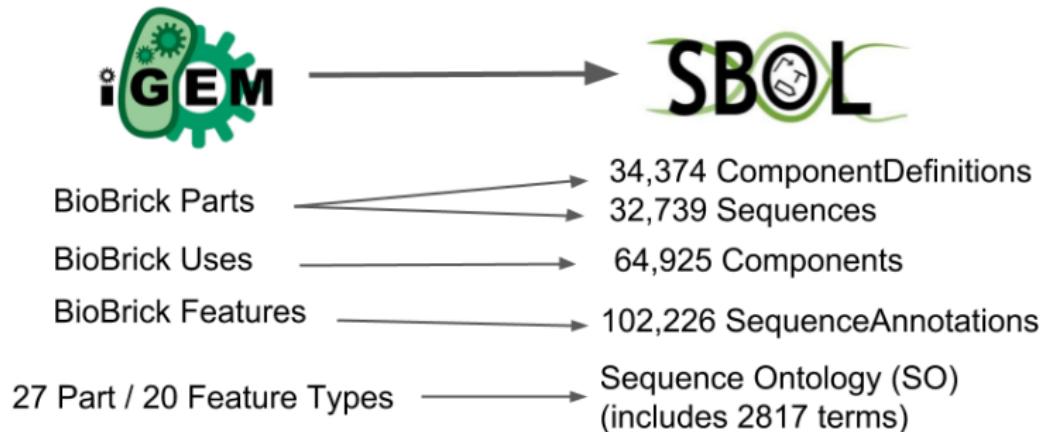


Conversion of the iGEM Registry to SBOL



| 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



Conversion of the iGEM Registry to SBOL



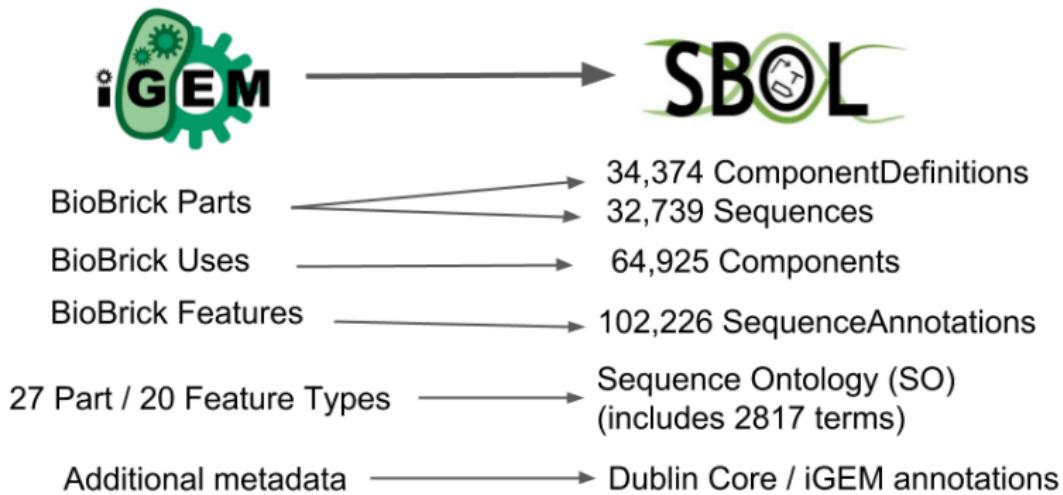
Page Header

[Login to edit](#)

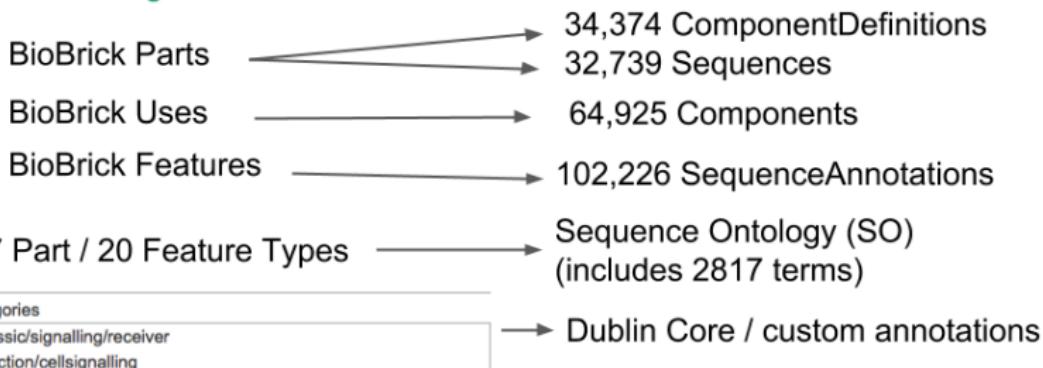
| | |
|------------------------|--|
| Part Name | BBA_F2620 |
| Short Description | 3OC6HSL → PoPS Receiver |
| Part Type | Signalling  |
| Nickname | |
| Designer(s) | Barry Canton [bcanton@mit.edu] and Anna Labno [labnoa@mit.edu] |
| DNA Status | Available |
| Qualitative Experience | Works |
| Group Favorite | No |
| Star Rating | 1 |
| Delete This Part | Not Deleted |

Dublin Core/iGEM Annotations

Conversion of the iGEM Registry to SBOL

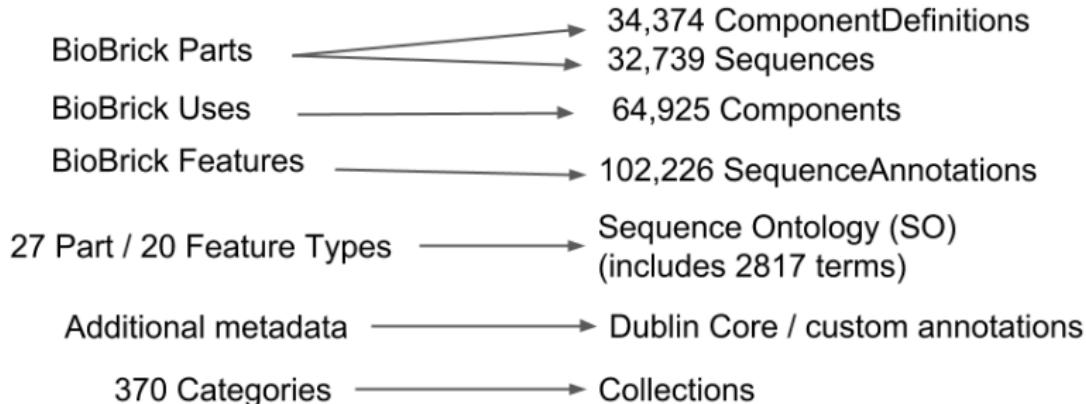


Conversion of the iGEM Registry to SBOL



Collections

Conversion of the iGEM Registry to SBOL



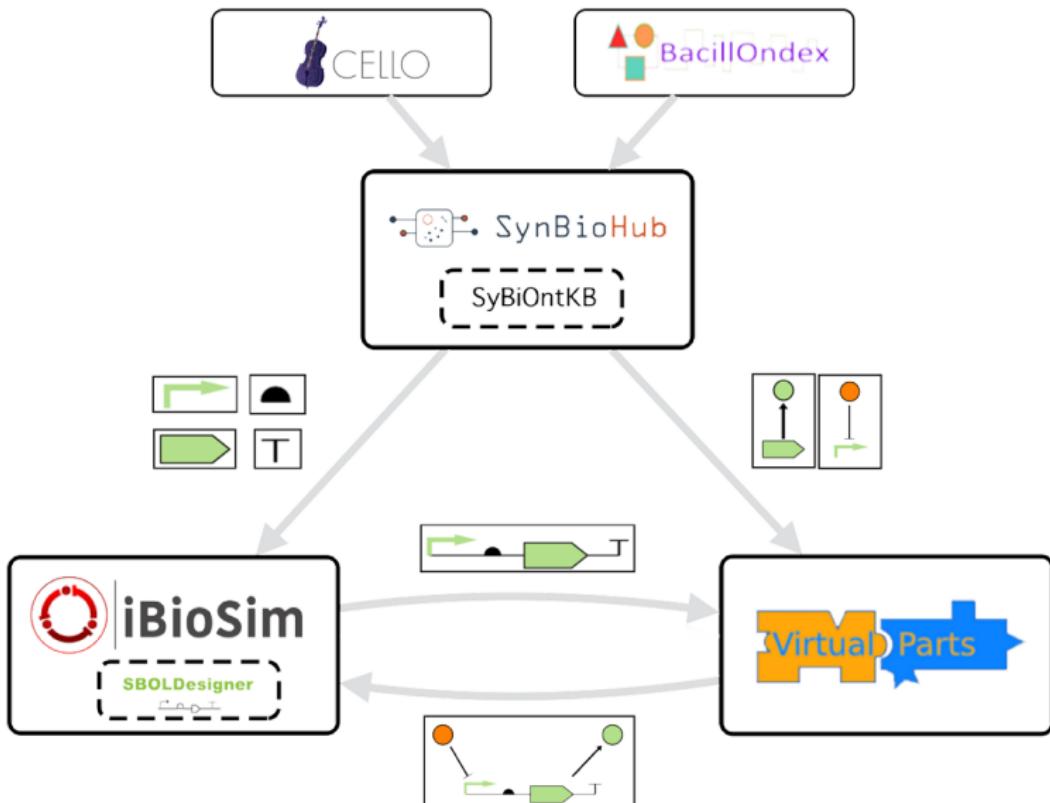
Observations

- Reuse
 - There are 64,925 documented cases of re-use.
 - There are 7,326 undocumented cases of re-use of sequences longer than 9 bases.
 - NOTE: only covers annotated features which have the same sequence as an existing BioBrick.
- Data inconsistencies
 - Found 834 instances of mis-aligned annotations.
 - Found 240 instances of basic BioBrick sequences not being consistent with their composite BioBrick sequences.

Additional Features of SBOL

- Tractable hierarchical representation of part-subpart relations.
- Systematic detection of inconsistencies in parts with SBOL tooling.
- Builds on standard ontologies, such as the Sequence Ontology.
- Enables powerful search queries using Semantic Web technology.
- Can convey versioning and rich provenance information via PROV-O.
- Enables users of the registry to take advantage of the emerging ecosystem of SBOL-enabled software tools.

Model Generation



Improving Synthetic Biology Communication: Recommended Practices for Visual Depiction and Digital Submission of Genetic Designs

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[§]DOE Joint Genome Institute, Walnut Creek, California 94598, United States

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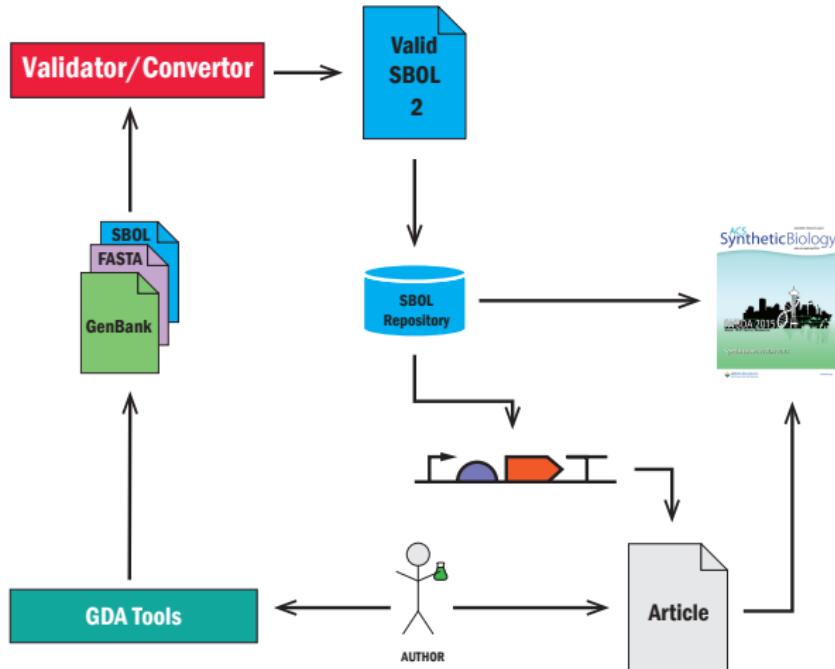
[⊥]Raytheon BBN Technologies, Cambridge, Massachusetts 02138, United States

[#]ACS Synthetic Biology, American Chemical Society, Washington, D.C. 20036, United States

ABSTRACT: Research is communicated more effectively and reproducibly when articles depict genetic designs consistently and fully disclose the complete sequences of all reported constructs. *ACS Synthetic Biology* is now providing authors with updated guidance and piloting a new tool and publication workflow that facilitate compliance with these recommended practices and standards for visual representation and data exchange.



ACS Synthetic Biology SBOL Workflow



Hillson et al., ACS Synthetic Biology (2016)
Zundel et al., ACS Synthetic Biology (2017)

libSBOLj 2.0 Released in Late 2015



Received 3 December 2015; accepted 27 February 2016. Date of publication 24 March 2016;
date of current version 14 April 2016.

Digital Object Identifier 10.1109/LLS.2016.2546546

libSBOLj 2.0: A Java Library to Support SBOL 2.0

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in part by the Engineering and Physical Sciences Research Council under Grant EP/J02175X/1.

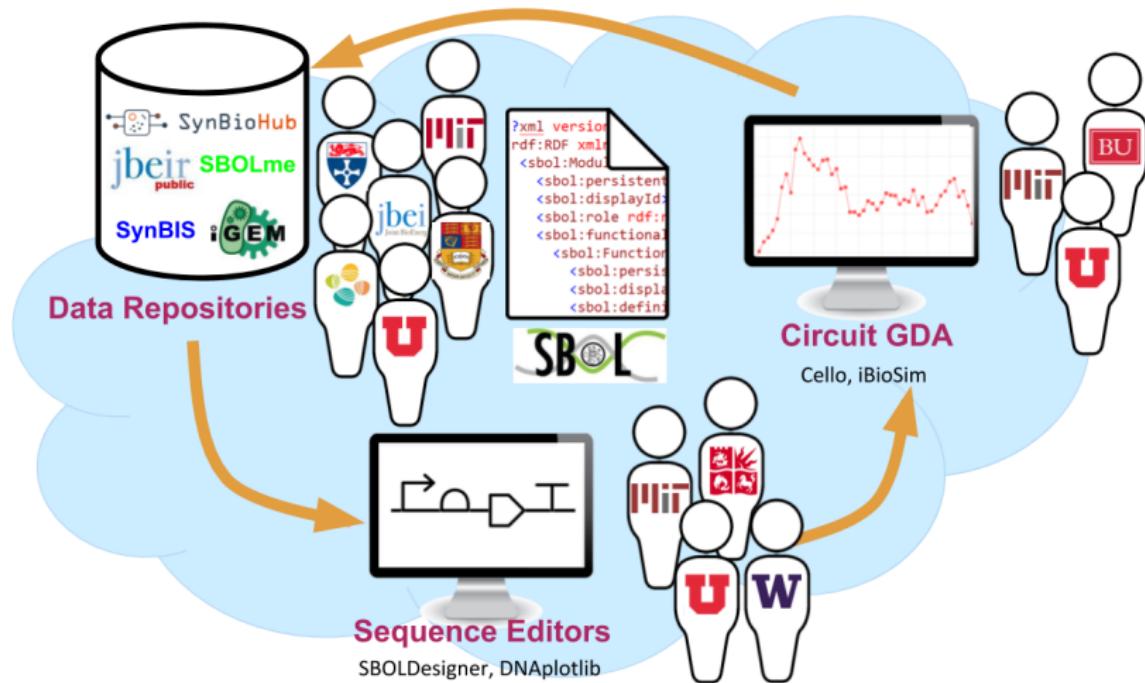
Other SBOL libraries available for C++, Python, and Javascript

Software Supporting SBOL

| Name | Function | | | | | SBOL | | |
|-------------------|----------|---|---|---|---|------|---|---|
| | R | S | V | G | M | 1 | 2 | v |
| Benchling | | • | | | | • | | |
| BOOST | | • | | | | • | • | |
| Cello | | | | • | | | • | |
| DeviceEditor | • | | • | | | • | | • |
| DNAPlotLib | | | • | | | • | | • |
| Eugene | • | | | | | • | | |
| Finch | • | • | | • | | | • | • |
| GenoCAD | • | • | | | | | | |
| GeneGenie | • | | | | | • | | |
| Graphviz | | • | | | | | | • |
| ICE | • | | • | | | • | • | • |
| iBioSim | • | • | | • | • | • | • | • |
| j5 | | • | | | | | | |
| MoSeC | • | | | | • | • | | |
| Pigeon | | • | | | | | | • |
| Pinecone | • | | | | | | | • |
| Pool Designer | • | | | | | • | • | |
| Proto BioCompiler | | • | • | • | | • | • | • |
| SBOLDesigner | • | • | | | | • | • | • |
| SBOLme | • | | | | | | | • |
| ShortBol | • | | | • | | | • | |
| SynBioHub | • | | • | | | • | • | • |
| Tellurium | | | | | • | | • | |
| TeselaGen | • | • | | | | • | | • |
| TinkerCell | | • | • | • | • | • | | • |
| VisBOL | | • | | | | | • | |
| VirtualParts | • | | | | • | | | • |

<http://sbolstandard.org/software/tools/>

Synthetic Biology Workflow Using SBOL



Data Repositories (SBOLme)



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العلوم والتكنولوجيا
King Abdullah University of
Science and Technology

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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

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.

Value

Pyruvate, C00022, ME_C00022

Search

25

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.

Data Repositories (ICE)



LIVING COMPUTING

Administrator

Search SEARCH

Create Entry

Featured 64

Personal 0

Shared 0

Drafts 0

Pending Approval 1

| Entry Type | Accession ID | Description | Status | Last Modified |
|------------|--------------|---|----------|---------------|
| PLASMID | LCP_000117 | pGOP35_pSB1C3-g13Op-minCMV-GFP-RBGpA The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Dec 2, 2016 |
| PLASMID | LCP_000116 | pGOP30_pSB1C3-g8Op-minCMV-GFP-RBGpA The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Dec 2, 2016 |
| PLASMID | LCP_000115 | pGOP25_pSB1C3-g3Op-minCMV-GFP-RBGpA The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Dec 2, 2016 |
| PLASMID | LCP_000079 | pGOP110_pSB1C3-g13Op_mismatch_1bp_loc10-minCMV-GFP-RB... The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Nov 28, 2016 |
| PLASMID | LCP_000078 | pOPP90_pSB1C3-g13Op_3multi_24bp-minCMV-GFP-RB... The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Nov 28, 2016 |
| PLASMID | LCP_000077 | pGOP85_pSB1C3_g13Op_2-multi-24bp_minCMV_GFP_R... The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Nov 28, 2016 |
| PLASMID | LCP_000076 | pGOP23_pSB1C3_g1Op_min-CMV_GFP_RBG-A The 2016 BostonU iGEM team designed a set of mutually orthogonal... | Complete | Nov 28, 2016 |
| PLASMID | LCP_000074 | pGEK108_pSB1C3_hU8_g8_SP-Cas9-pRNA-scaffold-RB... This part produces a guide RNA that pairs with an operator. This basi... | Complete | Nov 18, 2016 |
| PLASMID | LCP_000073 | pGEK103_pSB1C3_hU8_g1_SP-Cas9-pRNA-scaffold-RB... This part produces a guide RNA that pairs with an operator. This basi... | Complete | Nov 18, 2016 |
| STRAIN | LCP_000072 | Assembly #1 Recombinase_Level 1 Level 1 for assembly #1. | Complete | Nov 15, 2016 |
| STRAIN | LCP_000071 | gfp_pich41276_gi MoClo level 0 part. | Complete | Nov 15, 2016 |
| STRAIN | LCP_000070 | tp901_p-ag-bxbp-gt-tp901p-tc-eg Level 0 part. | Complete | Nov 15, 2016 |
| STRAIN | LCP_000069 | bfp_pich41258-de MoClo level 0 part. | Complete | Nov 15, 2016 |
| STRAIN | LCP_000068 | bxbp-gt_cd MoClo level 0 part. | Complete | Nov 15, 2016 |

NSF BOSTON UNIVERSITY MIT

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Ham et al., Nucleic Acid Research (2012)

Data Repositories (SynBioHub)



The screenshot shows the SynBioHub homepage. At the top, there's a navigation bar with icons for user profile, SynBioHub logo, "Submit", "About", "Submissions", and a power button. Below the header is a large banner featuring a DNA helix icon and the text "SynBioHub". A descriptive paragraph explains that SynBioHub is a design repository for biological constructs, enabling users to upload DNA and protein designs and search for existing parts. Below this are three main sections: "Search for useful parts and designs" with a magnifying glass icon, "Upload your design for safekeeping" with an upload icon, and "Share designs for publication or collaboration" with a share icon.

SynBioHub

Submit About Submissions

SynBioHub

SynBioHub is a *design repository* for people designing biological constructs. It enables DNA and protein designs to be uploaded, then provides a shareable link to allow others to view them. SynBioHub also facilitates searching for information about existing useful parts and designs by combining data from a variety of sources.

Search for useful parts and designs

Q. Gene or protein name Search

Browse Public Designs

Upload your design for safekeeping

Submit a Design

Share designs for publication or collaboration

Manage Submissions



James McLaughlin
Anil Wipat



Zach Zundel
Chris Myers

Version 1.0 released
June 14, 2017

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

[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



Living Computing Project

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

The screenshot shows the SynBioHub interface with a search bar and navigation links at the top. Below the search bar, there are five project cards, each with a logo, name, version, and a brief description. The projects are:

- 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.

Each project card includes a "PUBLIC" or "PRIVATE" badge with a red diagonal ribbon.

Sequence Editors (Benchling)

Other sequence editors that support SBOL:
**DeviceEditor, J5, VectorEditor (JBEI), DNAPlotLib (MIT/UW/Bristol),
Eugene (Boston), GenoCAD (VBI), BOOST (JGI), etc.**

Sequence Editors (SBOLDesigner)

The screenshot shows the SBOLDesigner software interface. At the top, there is a toolbar with various icons for file operations like new, open, save, and search. Below the toolbar, the title bar displays "GeneticInverter" and "Overview". The main workspace is titled "Design" and contains a schematic diagram of a genetic circuit. The circuit consists of a promoter (pAmtR), an attenuator (A1), a gene construct (BetI), and a terminator (L3S2P55). Below the schematic, the component names are labeled: pAmtR, A1, BetI, and L3S2P55. At the bottom of the workspace, there is a "Parts" section containing a grid of icons representing different biological parts: Gen, Pro, RBS, CDS, Ter, Ori, PBS, CUT, Scar, Op, Ins, RSE, PSE, RS, PS, BRS, _SOH, _3OH, and Circular.

Zhang et al., ACS Synthetic Biology (2017)

Circuit GDA Tools (Cello)

Cello Verilog Options Results About You are logged in as myers Logout

Verilog

```
1 module A(output out1, input in1, in2);
2 always#(in1,in2)
3 begin
4 case({in1,in2})
5 2'b00: {out1} = 1'b0;
6 2'b01: {out1} = 1'b0;
7 2'b10: {out1} = 1'b0;
8 2'b11: {out1} = 1'b1;
9 endcase
10 end
11 endmodule
12
```

Inputs

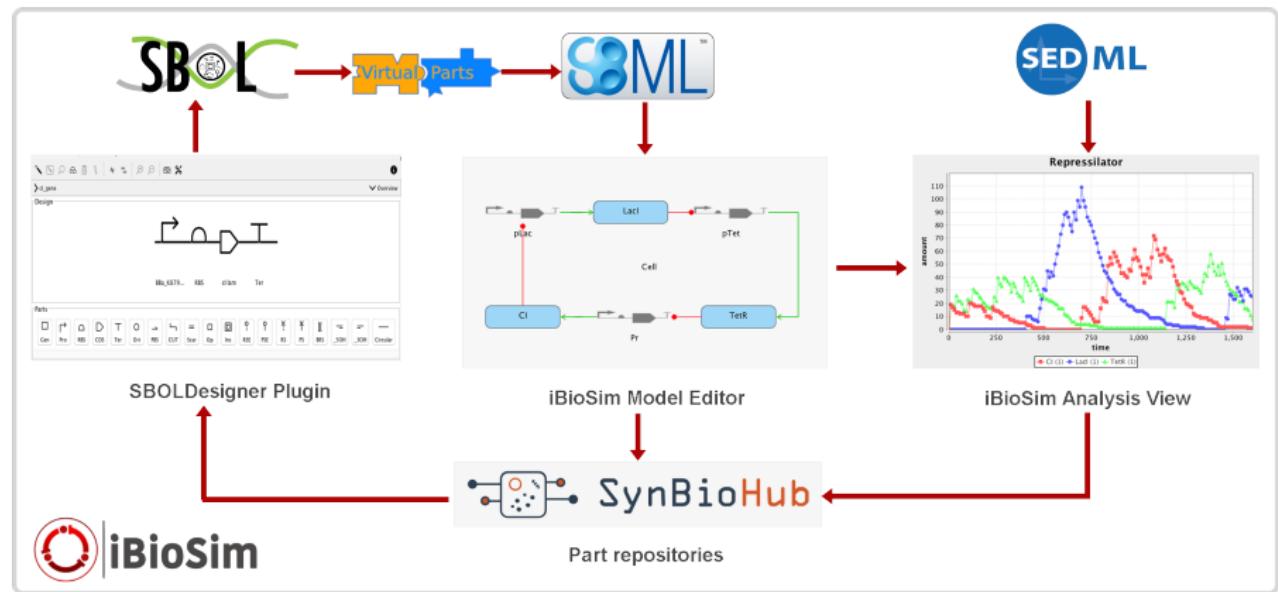
| index | name | low RPU | high RPU | DNA sequence |
|-------|------|---------|----------|--------------------------|
| 1 | pTac | 0.0034 | 2.8 | AACGATCGTTGGCTGTGTTGACAA |
| 2 | pTet | 0.0013 | 4.4 | TACTCCACCGTTGGCTTTTTCCC' |

Outputs

| index | name | DNA sequence |
|-------|------|--|
| 1 | YFP | CTGAAGCTGTCACCGGATGTGCTTCCGGCTGATGAGTCGT |

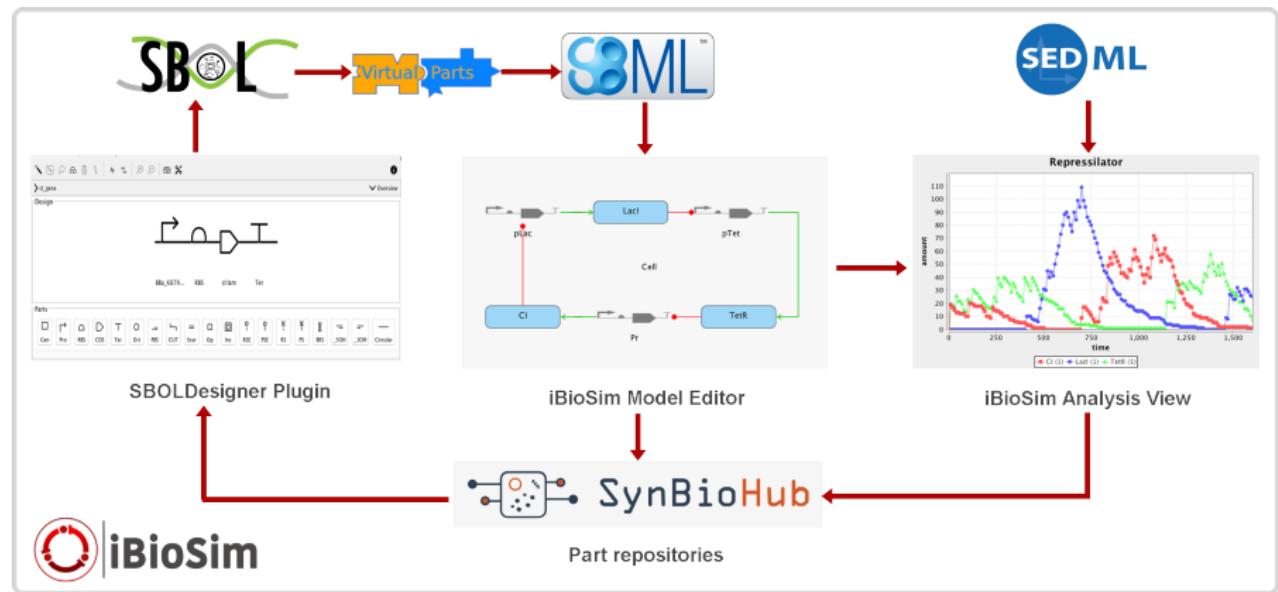
Nielsen et al., Science (2016)

Circuit GDA Tools (iBioSim)



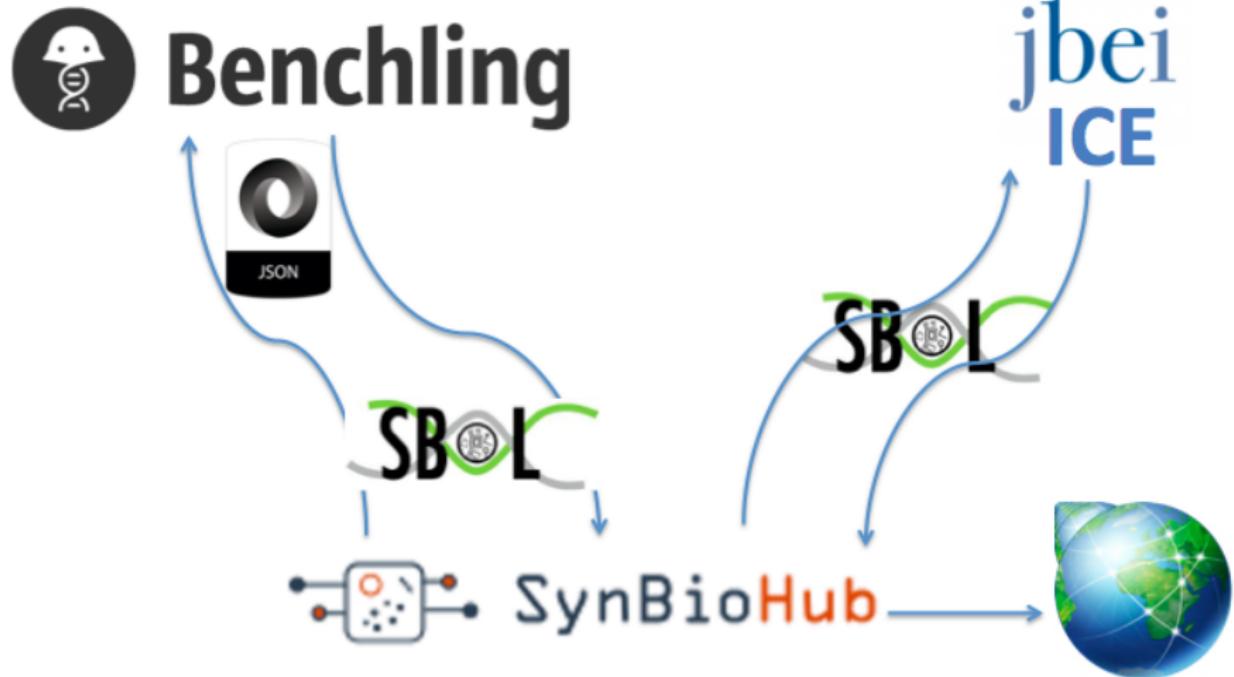
Madsen et al., IEEE Design & Test (2012)

Workflow Demo



Madsen et al., IEEE Design & Test (2012)

Workflow Demo



Workflow Demo

- This demo and much more information is available from:
<http://www.sbolstandard.org/>.
- Check out the SBOL Standard Youtube channel.
- SBOL is also on Facebook, Twitter, and Vimeo.

Workflow Demo