November 8, 2013

Dear Dr. Mak,

Apologies for the slight delay in resubmitting the revised manuscript. Seattle experienced a major power outage last night and even today the internet is not quite back to normal.

We would like to thank you and the reviewers for your valuable comments. In our revised manuscript, as suggested, we have completed a much more substantial demonstration of SBOL in actual deployment in a variety of applications at several academic and industrial locations both in the US and UK. This demonstration highlights both the ability of SBOL to enable exchange, as well as, the advantages that it has over related representations such as GenBank format. This exercise turned out to be very useful to us by highlighting some weakness in the tools which have now been resolved. Please see more details in our response to the reviewer comments below.

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Sincerely,



Herbert M Sauro  
Associate Professor  
Department of Bioengineering  
University of Washington

**RESPONSE TO REVIEWERS**

### *Reviewer #1*

1. In order to promote its use the utility of SBOL should and could be demonstrated on another less trivial example than the one demonstrated in this manuscript. The reviewer is not convinced in the first place that you need collaboration of 3 labs to perform the design of a single operon and secondly that the use of SBOL really helped to accomplish this simple task. The utility of SBOL standard will probably be apparent only in more complex constructs, where the success of SBOL supported abstraction will depend on the correct annotation of DNA components and their context (in)dependence.   
     
   **(Response) To address this concern, we have redone the SBOL demonstration to incorporate more tools on a more complex and realistic example. We are also providing more details about how SBOL facilitates the steps taken.**
2. While the context extension of SBOL is discussed with respect to the chassis and even used media it has not been described how the role of the context of specific DNA components will affect the function or how it should be defined/analyzed within the SBOL standard.  
     
   **(Response) It is clear that the behavior of DNA components is highly dependent on the context in which they are used, so models of the function of these components must take the context into account. This is clarified in the revised text as follows: “Devices gather components and sub-devices on the basis of shared function, while systems pair devices with their shared context. Models are associated with systems because the behavior of devices is closely tied to the context in which they are used. “**
3. The text is clearly written but could be substantially condensed to omit or move to the supplement some technical details such as in the section on the description of the standard. I suggest that this report could be published as a short report to make researchers aware of this standard.   
     
   **(Response) We have tried to remove repetition and tighten the text, but we are hesitant to move too much content to supplemental.**

### *Reviewer #2 (Remarks to the Author):*

1. I would like to see the introduction provide a bit more detail (perhaps after paragraph 1) about core characteristics of design in synthetic biology and the proposed workflows and abstraction hierarchies being pursued by practitioners, to better help contextualize the niche that SBOL is attempting to fill. There are several ideas introduced much later in the text (e.g. workflow, hierarchical design, DNA segment, device, system) that I think would benefit from a brief overview at the outset of the paper.

**(Response) To make the goals of SBOL more clear in the introduction, we have added the following text: “These requirements include the ability to describe partial or incomplete designs, as well as the capacity to create hierarchical designs that organize *DNA components* to achieve a desired function. Ultimately, synthetic biology workflows require the ability to encode additional information beyond an annotated sequence including, among other things, environmental and experimental context information, computational models of behavior, measurements of performance characteristics, etc. Therefore, a new, extensible standard is required to achieve these goals.”**

1. The authors describe one test case of exchanging data through SBOL among three institutions using three different tools. Given the number of institutions and applications that are mentioned as supporting SBOL (Table 1), I would have liked to see more done to demonstrate not just the potential but the actual deployment and ease of use of SBOL. Can the authors provide some indication of having tested SBOL across the range of applications listed in Table 1? And is there a clear process for users to report problems / request fixes from the Developer's Group during these early stages of testing and implementation?

**(Response) The contents of Table 1 are based upon self-reported information by the developers of these software tools and repositories. Unfortunately, with our volunteer effort, we have not had the resources to perform compliance testing though we hope to do so in the future. However, in our expanded SBOL demonstration in this revision, we have ensured that 5 software tools and 4 repositories from 6 institutions are able to exchange SBOL files. We have also added the following to the paper to point SBOL users to where they can report feedback:**

**“In order to provide feedback, report problems, and request features SBOL users can contact the SBOL Developers as described here:** [**http://www.sbolstandard.org/contact-us**](http://www.sbolstandard.org/contact-us)**”**

1. The section on 'Community' at this stage is more about the structure and governance of the SBOL Developers' Group, rather than a serious discussion of the role of standards in developing a synthetic biology community, or the means by which user requirements are being solicited and incorporated into SBOL core + extensions. This is fine but perhaps the section heading should change. I suspect the authors don't intend this, but this section instead risks giving a sense of predominantly one-way (top-down) relationships between SBOL and the synthetic biology community as opposed to deep two-way engagement. It presents the broader 'community' as somewhat external to SBOL, brought in to be the recipients of information that will hopefully build up 'excitement' and 'trust', rather than viewed as a key input to developing - and testing - a tool to serve the community. This could potentially have implications for the adoption and propagation of SBOL as a standard.

**(Response) We certainly did not intend to give the impression of a top-down development. To date, since SBOL has not been supported directly by any grant, it has been an entirely volunteer effort driven completely by interested members of the community. The SBOL Developers Group has a very broad representation of the community, and we are continuously seeking out new members to ensure that all constituencies are represented. This is critically important to ensuring that SBOL develops to be a standard that is both useful and used by the community. Another important point is the group is very open and democratic. Each participant has equal say. The SBOL chair and editors coordinate activities, but decisions are still made by the entire group. To emphasize these points, we have moved the Community section to right after the Introduction, renamed it to SBOL Developers Group, and rewrote this section to emphasize the open and democratic nature of our development process. We have also added an invitation at the end of the paper to new participants who would like to join in our efforts.**

1. In the final section (Future developments, limitations and conclusions) I would like to see the authors grapple more seriously with the possible limitations (or challenges) of this initiative - what are some of the core challenges to development, to sustaining funding and support for SBOL, and for securing community adoption of this standard? How does the group propose to facilitate adoption and propagation of this standard, and to support the refinement of SBOL? Is the hope that it will simply become a de facto standard through widespread uptake? I would also like a bit more specificity regarding the consultation and development process for SBOL - how widely has the Developers Group consulted with practitioners to develop a standard that meets existing (and desired future) needs? Or has this been a largely internal discussion?

**(Response) As described above, the revised paper adds more details about the workings of the SBOL Developers Group and how input is obtained and decisions are made. It is an open group representing a wide assortment of our constituencies that has enabled SBOL to overcome many of the challenges that you mentioned.**

1. Abstract - can it be said at this stage that SBOL is "community-adopted"? What evidence is there for this? Clearly this is a hope but the authors don't provide much empirical evidence for the extent to which it is widely used.

**(Response) The term “community-adopted” here refers to several things. The first is as you say the goal of SBOL is to be a standard for exchange that is widely supported. More than that, however, it also means that the standard is being developed in an open and democratic fashion. Anyone who wishes to participate in the development of SBOL is invited to do so, and that person will have equal say (“vote”) as every other member involved. All decisions for SBOL are made only after lengthy discussions concluding with a vote by all members of the SBOL Developers Group. While this group certainly does not include all synthetic biologists, it does include a very representative group in that there are both computational and experimental biologists, as well as, people from academic, government, and commercial organizations. Finally, community adoption has been highlighted by the fact that there now is significant support in both academic and commercial software. Also, a recent survey has shown that synthetic biologists are beginning to use SBOL. This is now described in the text as follows:**

**“The outreach efforts of the SBOL Developers Group have helped to attract early adopters. Recently 18 percent of self-identified synthetic biologists responding to a survey reported current use of SBOL and 10 percent past use17a, the highest use among standards and methods for measurement, functional composition, and data exchange in the survey. This base level of support forms a foundation for broader community adoption.”**

**Therefore, we have left this term in the abstract, but we have highlighted at the end of the paper that there is still a lot to do to further grow the level of community adoption, and we invite all to participate in these efforts.**

1. Introduction, line 1: 'biological organisms' instead of 'biological systems'?

**(Response) Changed.**

1. Introduction, line 3-4: replace 'to benefit society' with 'across a broad range of sectors'. It is hubristic to claim that synthetic biology offers nothing but benefits to society - this is as yet unproven and indeed all technologies come with both benefits and drawbacks.

**(Response) Changed.**

1. p.2, paragraph 2: standards have certainly been proposed for some measurements of biological parts, and for DNA assembly, but the way this is currently described makes these seem like settled and widely adopted standards. My understanding is that there are many alternative DNA assembly standards currently at play, and that remarkably few measurement standards have yet been proposed. So this might be better cast in more provisional terms.

**(Response) Changed to “Although standards have been proposed for experimentally measuring some key characteristics of synthetic biological parts12,13, 13a and for constructing composite DNA14, descriptions of the designs themselves have not been standardized.”**

1. p.2, paragraph 2: for a specific comparison of the differences between GenBank and SBOL 'file formats'?  
     
   **(Response) Added.**
2. p.2, paragraph 2: is synthetic biology more actively concerned with the design of novel DNA (and designing new function) than with its synthesis? Synthesis seems to be a necessary step but not really the focus of synthetic biology.

**(Response) Changed to “Synthetic biology is about the design of novel DNA to perform a desired function, rather than sequencing an extant molecule.”**

1. p.2, paragraph 5: It would be helpful to spell out the DNA sequence/segment difference somewhere in the main text as well as in Supplementary Table 1

**(Response) In the initial version, we misused the term DNA segment when we meant DNA component. This is now fixed, and we only mention DNA segment in this first paragraph in order to connect to this commonly using this term. We also now point here to the supplemental for the complete definition.**

1. p.3, paragraph 1: refer to preliminary 'testing'? It's not clear that on the basis of the single test described that the conclusion of 'broad benefit' has yet been actively demonstrated. Certainly there is promise, but the test as described does not really provide a sense of how easy and fast this process was, and such whether it is really likely to be widely adopted within the community.  
     
   **(Response) Changed “broad benefit” to “potential benefit”.**

1. p.3, paragraph 2: who ratified SBOL V1.1.0 and by what process?

**(Response) It was ratified by the SBOL Developers Group by a vote of the entire membership. This has been clarified at the beginning of this section.**

1. p.3, paragraph 2: what exactly is meant by 'toy'? Is the graphical representation (using SBOLv) done according to function?

**(Response) Changed “toy” to “simple”. SBOLv depicts the structure and not the function (at least currently). So, we’ve changed the caption to: “SBOL Visual20 enables the depiction of the structure of genetic designs in a standard graphical notation.”**

1. p.3, paragraph 3: again, how were the 'stakeholder goals' developed and how many stakeholders/ who do they represent?

**(Response) The goals are set through both email discussions on our mailing list, as well as, face-to-face workshops. This process is detailed in the revised SBOL Developers Group section. The stakeholders are described in the revised version at the beginning of this section as follows: “SBOL has been under development by the SBOL Developers Group, a diverse group of both experimental and computational synthetic biologists from academic, government, and commercial organizations, since 2008. At this writing, the SBOL community has 76 delegates from 37 organizations (23 academic, 11 commercial, 2 government labs, and 1 independent), who work across organizational and international boundaries to set priorities and reach agreement on the standard. Any practitioner may join the SBOL Developers Group, and we are continuously reaching out to attract new members to broaden the representation of the synthetic biology community within the group.”**

1. p.4, paragraph 2: 'the synthetic biology workflow' - a bit more information about workflows and abstraction hierarchies earlier in the paper would help contextualize the need / possible contributions of these proposed extensions

**(Response) As described above, we have added a bit more to the introduction to explain the workflow needs that SBOL is trying to address. We have also updated the SBOL demonstration to illustrate more steps of the complete synthetic biology workflow. Namely, we start by constructing an abstract partial design using parts from a repository. Next, we complete cassettes by defining the remaining components producing several design permutations. From these cassettes, we construct a complete genetic toggle circuit and associate models with the circuit. Finally, the resulting complete design is imported into Life Technologies VectorNTI tool to optimize the sequence before synthesis, and it is deposited in multiple repositories.**

1. p.4, paragraph 4: how was the list of variables for environmental context derived?

**(Response) Small working groups are developing the extensions, but all extensions are finally ratified by the entire SBOL Developers Group to ensure that all requirements are met. The following text has been added to the description of the extensions to make this process clear: “These extensions are being developed by small working groups within the SBOL Developers Group. Ultimately, specifications for them will be presented to and ratified by the entire group.”**

1. p.5, paragraph 1: do these extensions fall more appropriately into a 'future developments' section? Can the authors provide a bit more clarity on the process by which these extensions are considered and developed?

**(Response) This has been moved into the future work section. Text has been added to this section to describe their development and ratification process.**

1. p.5, paragraph 3: "they selected three components" - for clarity, specify which components. This paragraph describes the sequential transfer of information across institutions, but makes no reference to the ease or timescale with which this was done, or whether this test suggested any fixes or improvements that could be made to facilitate data transfer. Has the full range of SBOL-compatible software tools been tested in this way? It would be nice to see more evidence of SBOL use across a range of tools, in order to better demonstrate its broad applicability and usefulness.  
     
   **(Response) We have added a more practical and detailed demonstration of SBOL involving more software tools from a variety of institutions. We have also made available all the SBOL files exchanged in our Supplemental Materials. The testing of SBOL compliance of software tools is one area of work for which we have been seeking funding.**
2. p.5: perhaps Figure 1(b) would be better off as a figure in its own right.

**(Response) This makes sense. We’ve split Figure 1 into two separate figures as requested.**

1. p.6: I would swap the order of paragraphs 2 and 3.

**(Response) Swapped.**

1. p.6, current paragraph 2: what are the 'lessons learned' from SBML, and are these documented/ referenced anywhere? I'm not sure that this is how the authors intend it, but this paragraph reads more as a top-down push by the developers' community to promote SBOL, rather than a genuinely community-led initiative to solicit input and respond to community wishes and needs.  
     
   **(Response) The text has been revised to make the lessons learned from SBML clearer. Namely, the need to have an open and democratic organization, as well as, engaging young researchers and having bi-annual meetings.**
2. p.6, paragraph 4: an email address or contact information for submitting requests to the SBOL editors/developers would be useful to provide

**(Response) We’ve added an invitation, with an email address for the editors, to join the SBOL developers group.**

1. Table 1 and Supplementary Table 1: listing entries in alphabetical order would be more helpful (as it stands they are not even in order of appearance in the main text)

**(Response) Fixed.**

1. Supplementary Table 1 - entry for 'standards': I'm not sure I agree with the statement that all standards increase industrial and scientific productivity; some certainly turn out to be counterproductive. And it's not clear that improvements in e.g. 'quality' or 'safety' (issues that many standards focus on) are necessarily equivalent to improvements in 'productivity'  
     
   **(Response) Changed to “Set of agreed upon and adhered to technical definitions and guidelines created with the goal to increase industrial and scientific productivity1.”**
2. Supplementary Table 1 - entry for SBOL: is the data standard simply for exchanging designs? Or is it a way of representing designs that is compatible with a variety of application tools, and tailored for easy exchange?

**(Response) Changed to “SBOL is a data standard in synthetic biology used to represent designs in such a way that is compatible with a variety of applications to foster easy exchange.”**

1. Supplementary Table 1 - entry for 'DNA segments': do you mean 'segments' instead of 'regions'?  
     
   **(Response) Yes, changed.**
2. Supplementary Table 2 - the second row doesn't seem to be entirely comparable across entries  
     
   **(Response) Changed row to reflect goals and membership of both organizations. Changed SBOL entry to “Development and maintenance by the SBOL Developers Group, to support data exchange among synthetic biologists, the software tools they use, and repositories. Membership is open to all affected interests.“ Clarified GenBank format entry, “to support aggregation in centralized databases. Consortium is made up of members of each of the databases' advisory bodies.”**

### *Reviewer #3 (Remarks to the Author):*

1. The question of originality is a little complex in that the paper brings together work and tools from a number of different groups many of which have been used previously. The key to this paper is their integration via the new version of SBOL. In my opinion the authors could clarify further how the new version improves on the old version. In addition much of the work described in this paper has appeared in a BioBricks Foundation request for comments published online. This should not necessarily preclude publication in Nature Biotechnology but should be noted.  
     
   **(Response) Unfortunately, there was a little confusion in that this paper is not meant as a description of a new version of SBOL, but rather an introduction of SBOL in general. Indeed, this is the first publication about the SBOL standard. The confusion is perhaps due to the fact that the SBOL version moved from 1.0 to 1.1 during the preparation of this manuscript to correct a couple of issues discovered as people started using 1.0. We have clarified the SBOL versioning in the paper. We have also made it clear in the introduction that we are describing SBOL in this paper and not just the changes from 1.0 to 1.1.**
2. The approach is absolutely justified and the presentation is of good quality. There is not much data as such presented - this is not a standard research paper. While I appreciate that the aim of this paper is to bring together different designs and tools it would have been more convincing if the authors had actually synthesized the designed device and tested it in a biological context to validate their methodology.

**(Response) While we feel that testing in the lab is outside the scope of this paper, we do feel the demonstration needed to be more realistic and involve more groups and software tools. To this end, we have completely redone the demonstration as described in the paper and Supplemental Materials to better highlight the strengths of SBOL for data exchange over alternatives such as the GenBank format.**

1. The conclusions are robust. This is an extremely important community effort of great value to the field of synthetic biology. Such underpinning standardization and development of ease of data and design exchange are vital if the field is to achieve its potential in the engineering of biology. I am convinced that SBOL will be the accepted standard for the Synthetic Biology community and will continue to be developed and evolve new applications and extensions. As far as this paper is concerned I would have liked to have seen more discussion on any competing standards and more discussion contextualizing it with other standards from other communities (I know that systems biology standards were mentioned briefly). The comparison with Genbank is interesting but limited.

**(Response) There are indeed many other related standards out there, though we would not say they are competing necessarily. Given our limited resources, we must be careful to leverage these other standardization efforts, whenever possible. Examples mentioned in the original submission included SBML, SO, and SBO that we are already utilizing. We also plan to leverage BioPax for describing interaction pathways, and SBGN for graphical representations. These additional standards are now also mentioned in the modeling extension section. In order that these standards meet our needs, it is critical that we better coordinate our efforts with those working on these related standards. This fact is now mentioned in the conclusion as one of our future challenges.**

1. The paper was clear and over all very well written but a more information in the text to briefly explain what tools such as Proto Biocompiler and iBioSim would help the non-expert reader. The final paragraph on page 3 is written in a very dense manner. Page 5 - the phrase "The Boston team added additional sequence information needed for construction" is too vague. What was the additional sequence information? The paper could be tightened up by removing some of the repetition - the aims of SBOL are reiterated a little too often.

**(Response) The SBOL demonstration has been expanded to make it both more practical but also more detailed. It also now involves more software tools from a wider variety of institutions.**

1. The paper has been submitted as a "perspective". I am not convinced that it fulfills the criteria for a perspective as described on the Nature Biotechnology website. It is not really a discussion of the primary literature and there is no discussion of a controversial area. However, I do think SBOL is an important standard and should be published and Nature Biotechnology is the most appropriate home for it to reach a broad audience of scientists already engaged in Synthetic Biology and also those who are interested and will become more engaged as the field matures and more tools become available.

**(Response) As requested by the editor, we have ignored this comment.**