

Standardization in Synthetic Biology

Kristian M. Müller and Katja M. Arndt

Abstract

Synthetic Biology is founded on the idea that complex biological systems are built most effectively when the task is divided in abstracted layers and all required components are readily available and well-described. This requires interdisciplinary collaboration at several levels and a common understanding of the functioning of each component. Standardization of the physical composition and the description of each part is required as well as a controlled vocabulary to aid design and ensure interoperability. Here, we describe standardization initiatives from several disciplines, which can contribute to Synthetic Biology. We provide examples of the concerted standardization efforts of the BioBricks Foundation comprising the request for comments (RFC) and the Registry of Standardized Biological parts as well as the international Genetically Engineered Machine (iGEM) competition.

Key words: Biobricks, Biological parts, iGEM, Registry, Standard

1. Introduction

1.1. Development and Scope of Synthetic Biology

A short look into history provides a basis for the understanding of the scope of standardization in Synthetic Biology. The term Synthetic Biology has been phrased several times and evolved along the development of natural sciences. In the early twentieth century molecular disassembly of life took off and biochemists, such as Jaques Loeb, declared the “abiogenesis” as research goal. In 1912, Stéphane Leduc from France published a book titled “La Biologie Synthétique” (1). In the 1970s, the genetic revolution started and an editorial of *Gene* stated in 1978: “into the new era of ‘Synthetic Biology’ where not only existing genes are described and analyzed but also new gene arrangements can be constructed” (2). Yet, in both historic events, the term Synthetic Biology did not make it into scientific mainstream. The terms “biochemistry” or “physiologic chemistry” and later “molecular biology” and “genetic engineering” better described

the research and technology of that time. Finally, at the end of the twentieth century, biologists and engineers again were searching for a new name for their technological ambitions to engineer biological systems. Many aspects of bioscience had matured, and after about 10 years the term Synthetic Biology is finding widespread acceptance. It describes an interdisciplinary approach, which is nurtured from many scientific sources. One uniting theme is a quote attributed to Richard Feynman “What I cannot create, I do not understand,” which appeals to researchers and engineers alike. A workgroup initiated by the European Commission developed the definition: “Synthetic Biology aims to engineer and study biological systems that do not exist as such in nature, and use this approach for (i) achieving better understanding of life processes, (ii) generating and assembling functional modular components, and (iii) developing novel applications or processes” (3).

This open view appeals many protagonists. At the same time, it precludes an exhaustive description of all potential standards. This chapter touches standardization as seen in nature (Subheading 2), biology (Subheading 3.1), non-clinical and clinical research (Subheading 3.2), engineering (Subheading 4), and last but not least Synthetic Biology (Subheading 5). Strategies enabling language control and electronic processing are covered (Subheading 6).

1.2. Standards and Standard Setting

Setting standards can serve several purposes. Standards can improve communication, compatibility, interchangeability, reproducibility, effective use, fitness for use, safety, quality assurance, and ultimately consumer protection and environmental protection. Standards can be grouped by type. Technical standards provide specifications, which could be input and output values, procedural standards may describe a workflow, which can be standard operating procedures, and classification standards may provide definitions, which can be a controlled vocabulary. Standards can be grouped by accessibility. Proprietary standards are not freely available, whereas open standards are freely available. In a more narrow definition, a “real” standard has to be freely available. The connotation open standard emphasizes this point and describes a standard which is easily accessible and can be further developed by a wider community. Standards can be described by their development and requirements for use. Standards can be voluntary or mandatory. A de facto standard is a convention accepted by a wider public. A de jure standard is enforced by governments.

A standard is typically described in a document and a standardized material adheres to production or specification standards. In contrast, a standard reference material is a controlled artifact used for calibration.

The standard setting process can be manifold. Standards can emerge unintended as a result of usage habits. They can be set by standards organizations or bodies often affiliated with a state or by

standard-developing organizations. Rules and goals of standards vary widely with respect to stakeholders, beneficiaries, formal process, and voting process. In basic research, standards are typically a community effort and users vote with their feet. In clinic studies, governmental regulation prevails.

2. Standardization in Nature

2.1. Scale of Natural Standards

Although one might think of nature as wild, untamed, and chaotic, it should be noted that nature evolved standards and solved compatibility issues over space, scale, and time, which humans never will surpass. From a single-cell organism, such as *E. coli*, with a length of about $1\text{ }\mu\text{m}$ to a blue whale with a length of about $3 \times 10^7\text{ }\mu\text{m}$ or the human brain with more than 5×10^{10} cells, nature uses the same set of building blocks of (deoxy-)ribonucleic acids, amino acids, sugars, and lipids. The basics of this standard set emerged about 1.7–2.5 billion years ago when the common ancestor of cellular life diverged into the kingdoms of life. Many building blocks and metabolic processes as we know them today are stably implemented since hundreds of millions of years. Building blocks enabling self-assembling over billion-fold size and data storage for the construction of these building blocks over more than billion years are at present beyond imagination for human technology and provide the gold standard for any sustainable technology. The compatibility between organisms is nicely demonstrated when a gene from a jelly fish is plugged into a bacterium or human cell resulting in the expression of a fluorescent protein. A more natural but less-desired example of compatibility is the rapid spread of antibiotic resistance among microbial pathogens. This admiration for the results of evolution does not preclude attempts to further standardize biological components and systems for current engineering needs in Synthetic Biology.

2.2. Reasons for Natural Standards

Standardization seems to be a human idea rooted in intelligent perception and technical mastery. However, when it comes to biology, standardization can be seen as a necessity of life. Sustaining life on earth requires the endless recycling of all chemical compounds required for life. It is fair to assume that recycling is most energy efficient if all life forms use a similar or even the same set of components and, as a consequence, life that deviates too much from the common chemical basis will sooner or later become extinct. A second argument for the observed standardization in biology is the low likelihood of invention of new functional entities. The latter argument was overcome by humans, who began to create “electronic life” and run the large-scale experiment of how biologic and “electronic life” can coexist.

2.3. Abstraction in Nature

One major goal of standardization is to provide components with defined input and output characteristics that can be used without hassling with the details of each subsystem. At the organismic level, this works perfectly well and even stone-age people were able to handle organisms in an abstracted fashion. They added soil and water as input to crop seeds, which are the gray box, and harvested the output. Furthermore, no biological education is required to combine two interspecies abstraction layers, for example by using the “crop-system” output as “cow-system” input to obtain milk. To some extent, biology’s intrinsic compatibility can be used at the genetic level by plugging a gene from one organism into another. However, typically above the layer of basic building blocks and the very similar use of the genetic code, compatibility breaks down. This is even true when organisms use the same principles for metabolism and signaling. At the long-known organismic level, this is seen by the fact that breeding a horse with a horse gives propagable offspring, breeding a horse and a donkey gives almost exclusively infertile offspring, and breeding a horse with a cow does not work. This is why setting standards at the molecular biology level is a major task.

3. Standardization in Biological Research and Development

3.1. Standardization in Basic Biological Research

With increasing amounts of biological data and even more with the advent of large-scale data generation experiments, it became apparent that the diversity and incompleteness of classical method publishing provides a roadblock for data comparison and data processing. Electronic databases curated by teams also functioned as nucleation factor for standards in reporting methods and experimental results. A discipline, which early adopted rules of data reporting and submission to a database, was structural biology, namely, crystallography. A formal guideline from 1989 was widely adopted also by publishers (4). DNA sequence data were also among the first deposited in databases (5). In this case, only the final sequence was stored and recently new reporting standards in genomics were demanded (6).

In the meantime, several life science subdisciplines realized the need for traceable experiments and standardized sharing of information and consequently established rules to report experiments. One umbrella effort is the “Minimum Information about a Biomedical or Biological Investigation” (MIBBI) foundry (<http://mibbi.org/>) (7). An early effort was aimed at the comparability of microarray experiments (MIAME) (8).

A prominent set of rules with implications for Synthetic Biology has been established by the Human Proteome Organization (HUPO, <http://www.hupo.org>). These guidelines specifically include the proteomics aspect of an experiment and are, thus,

named minimal information about a proteomics experiment (MIAPE) (9). For standardization, the HUPO's Proteomics Standards Initiative (HUPO-PSI) has developed a formal process for setting guidelines (10). Within the MIAPE guidelines, there are further detailed guidelines, such as for the reporting of the use of gel electrophoresis (11), for reporting a molecular interaction experiment (MIMIX) (12), or reporting the use of mass spectrometry (13). Further rules are established for experiments, such as chromatography (14), proteomics images (15), and proteomics capillary electrophoresis (16).

3.2. Standardization of Research in Non-clinical and Clinical Research

Human health is a major concern for governments, and thus in research and development aiming at substance release or human therapy adherence to standards is required by law. A well-known standard is the "good laboratory practice" (GLP) applying to regulated non-clinical research and development. Requirements are coded, e.g., in the USA in the Code of Federal Regulations (CFR), by European Union directives, and by the OECD. According to the OECD GLP principles, which acquired wide international acceptance, GLP is a "a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported" (17).

A further standard widespread in pharmacological research is the pharmacopeia, which is a reference work composed of monographs describing substances, materials, and dosage forms. For example, the European Pharmacopoeia (Ph. Eur.) is maintained by the European Directorate for the Quality of Medicines and HealthCare (EDQM), which is a Directorate of the Council of Europe (<http://www.edqm.eu>). EDQM also provides the International Standards for Antibiotics (ISA) and is responsible for the establishment and distribution of WHO's International Chemical Reference Standards (ICRS). Unfortunately, online access has to be purchased.

Standardization efforts have also been developed for clinical experiments. The "Consolidated Standards of Reporting Trials" (CONSORT, <http://www.consort-statement.org/>) comprises several initiatives to avoid problems arising from inadequate reporting of randomized, controlled trials (18).

3.2.1. Recording Data According to GLP

The following protocol is adapted from ref. 17 with minor modifications to ensure correctness. Data are necessary for the reconstruction of the study after completion.

The following items have to be recorded:

1. WHAT was done: A description of the conduct of the technique, including the results of the observation or measurement, and demonstrating that the actions required by the protocol were carried out.

2. HOW it was done: The data should indicate that they were collected and recorded in accordance with the methods set out in the standard operating procedures (SOPs) and protocol or indicate where there were deviations from these instructions.
3. WHEN the work was performed: A demonstration of compliance with the schedule defined in the protocol. This is done by recording the date and, if necessary, the time that the procedure was conducted (see Note 1).
4. WHO performed the work: The data should clearly identify who was responsible for carrying out the procedure and recording the data. If more than one person was involved in a procedure, this should be recorded in the data, along with an identification of the responsibilities of each.

The generated data should be recorded as follows:

1. Directly: The first written records are considered to constitute the raw data and must be retained. Records should not be made on scraps of paper and then transcribed into a final form. When data are acquired directly by computer, the raw data are considered to be the electronic medium. For data derived from equipment, the raw data may be a direct printout (or trace) or in an electronic form.
2. Promptly: Data must be recorded as the operation is done. It is not acceptable to make the record sometime after the task has been completed.
3. Accurately: This is most important as the accuracy underpins the scientific interpretation and integrity of the study.
4. Legibly: Data that cannot be read are useless, and records that are difficult to decipher raise doubts as to their credibility.
5. Indelibly: Only indelible and waterproof pens and robust machine printouts are permitted. In case of question, additional authorized photocopies might be used for storage.

In addition, the data need to be:

6. Identified: The study number, animal number, etc. must be recorded with the data in order to ensure that data mix-ups do not occur. The parameter evaluated must be identified.
7. Signed: Accountability is one of the basic tenets of GLP, hence the need for a record of who did every job on a study.
8. Dated: The date of each signature demonstrates that the procedure was conducted and recorded at the correct point in the study.
9. Corrected only with given reasons: Records may require alteration from time to time, but a clear audit trail is needed showing why a change was carried out, when and by whom.

Data should be recorded and organized in a way that facilitates both the making of the record and the performance of subsequent processes (e.g., data entry, reporting, audit, archiving). Data should be recorded in a logical way, and duplication should be avoided wherever possible. Preformatted documents assist in this by encouraging staff to record all the data required, without forgetting any. A clear structure for the study file, defined upfront, helps to organize and archive the documents as they are produced in real time, preventing loss and facilitating reference between records.

If computerized systems are used to generate or process data, correct operation of data handling and safe storage of the system have to be validated.

4. Standardization in Technical Disciplines

4.1. Comparison of Synthetic Biology with Mechanic and Electrical Engineering

In the realm of Synthetic Biology, the commercial success of mechanical engineering and electronics has often attributed to standardization and has been praised as the desired goal of Synthetic Biology. In early days of the reincarnation of Synthetic Biology, cells have been depicted as bags of screws, in which just the thread of the screws has to be adjusted to obtain an easy-to-manage system. This primitive view was probably necessary to get the attention of engineers, although it frustrated biologists who abandoned this scheme decades ago. Above the screw, the transistor and the many commercial, well-defined electronic parts were used to symbolize standardization. Plugging genes in a genome, like transistors on a circuit board, is a tempting idea, but unfortunately countermanded by the next level of comparison. At present, many Synthetic Biology talks and some publications compare a cell with a computer (19). Here, a physical layer of, e.g., proteins, is compared to transistors, and biochemical reactions are compared to gates and pathways to modules, such as integrated circuits. The good aspect of this comparison is that biologists and engineers can agree on such an analogy. The bad news is that this comparison leaves little room for off-the-shelf standard parts. Computer chip development is in reach only for a few companies worldwide, and production takes place in foundries costing billions of dollar/euro to set up. At the same time, the typical life span of a computer chip series, which requires highly adapted boards and brides for its life cycle, is about 2 years. Synthetic Biology parts, which become incompatible within the life span of mouse, are unattractive. Within this comparison, Synthetic Biology should position its standards between the off-the-shelf transistor and the modern computer industry.

Even if engineering does not provide a gold standard, Synthetic Biology has much to learn from these disciplines. Foremost, this is the divide and conquers approach to a complex problem by defining abstraction layers, a working set of conditions and specifications for each required part. Most impressive to biologists is the separation of hard- and software in the computer industry. And the soon-to-come programmer of an organism cannot be knowledgeable about all the enzymatic details in a cell.

Another role model of technical engineering is public acceptance. If engineers would develop standards, which would allow cars to drive people twice as fast to work as today, most people would cheer the development. If synthetic biologist would develop strategies that make horses run twice as fast to work, most people would be scared. Thus, the process of setting biological standards has to take into account public opinion and norms, which often entail a love for nature conservatism and to some extent biophobia.

4.2. Standardization According to ISO

A well-known organization developing technical standards is the “International Organization for Standardization” (ISO, <http://www.iso.org>). Although standards published by ISO are widely adopted by the industry because legislative bodies tend to encourage their use, they are not available for many researchers due to the costs for the documents. Some ISO publications provide rules, which could be of interest to scientists. For example, ISO 11737–1:2006 “specifies requirements and provides guidance for the enumeration and microbial characterization of the population of viable micro-organisms on or in a medical device, component, raw material or package.” A standard published by the ISO has to pass several hurdles. From the first concept to publication, the following statuses might apply: Preliminary Work Item, New Proposal/New Work Item Proposal, Approved New Work Item, Working Draft, Committee Draft, Final Committee Draft, Draft International Standard, Final Draft International Standard, Proof of a new International Standard, and International Standard. Taken together, these standards are useful for specific tests, but the ISO process of defining standards seems not suited for Synthetic Biology.

4.3. Standardization for the Internet

For outsiders, it may seem off topic to discuss standardization of the Internet community. However, within the Synthetic Biology community, the fast-paced development of hardware, software, and the Internet is seen as a paradigm for success. In addition, Randy Rettberg, the prominent figure behind iGEM, has ties to the Internet development. Hence, it is worthwhile to take a look at this example. The Internet is mainly standardized by the “Internet Engineering Task Force” (IETF, <http://www.ietf.org>), which publishes memorandums named RFC. RFCs cover a wide range of topics comprising methods, behaviors, research, or innovations related to the Internet and its connected systems. The basic

definition of the IETF standards process is described in RFC 2026 and its amendments. One section of this RFC is highlighted on the IETF Web site (<http://www.ietf.org/rfc/rfc2026.txt>) and is given below, as it fits to the interests and problems of Synthetic Biology (see Subheading 5.1):

“In outline, the process of creating an Internet Standard is straightforward: a specification undergoes a period of development and several iterations of review by the Internet community and revision based upon experience, is adopted as a Standard by the appropriate body [...] and is published. In practice, the process is more complicated, due to (i) the difficulty of creating specifications of high technical quality; (ii) the need to consider the interests of all of the affected parties; (iii) the importance of establishing wide-spread community consensus; and (iv) the difficulty of evaluating the utility of a particular specification for the Internet community [...] The goals of the Internet Standards Process are (i) technical excellence; (ii) prior implementation and testing; (iii) clear, concise, and easily understood documentation; (iv) openness and fairness; and (v) timeliness.

[...] The goal of technical competence, the requirement for prior implementation and testing, and the need to allow all interested parties to comment all require significant time and effort. On the other hand, today’s rapid development of networking technology demands timely development of standards. The Internet Standards Process is intended to balance these conflicting goals. The process is believed to be as short and simple as possible without sacrificing technical excellence, thorough testing before adoption of a standard, or openness and fairness.”

The IETF RFCs can have increasing degrees of importance. The status can range from “informational,” “experimental,” “best current practice,” and “standards track.” Standards track documents are further divided into “proposed standard,” “draft standard,” and “Internet standard document.” Documents, which become obsolete, may get the status “historic.”

5. Standardization in Synthetic Biology

5.1. Overview

Since Synthetic Biology builds on several life science disciplines and adds its own dimension, it also requires a broad set of standards to enable productive collaboration. Biology alone encompasses diverse samples ranging from small molecules to ecosystems and diverse technologies ranging from biophysics to behavioral analyses. The bold goals of Synthetic Biology aim at providing effective solutions for health, agriculture, renewable resources, and energy based on bioengineering and bioinspired engineering. The current status of Synthetic Biology can still be seen as the enabling technology

build-up phase. Most efforts focus on establishing basic genetic engineering in a standardized fashion and demonstrating understanding of biology by bottom-up engineering. In contrast to purely man-made technology, often manifold examples of potential solutions can be found in natural systems; yet harnessing, tweaking, and managing these natural solutions provide a formidable challenge. Next to genetic engineering comes the engineering of other biomolecules, most notably protein engineering. Depending on the set goal, requirements for standardization can vary significantly. For example, the current challenge for biopharmaceuticals often falls within protein engineering while providing future solutions for regenerative medicine requires manipulation of cells and organs. As a consequence of this diversity, Synthetic Biology heavily relies on standards developed in various disciplines. The main task for Synthetic Biology is to clarify the abstraction hierarchies and rules of how to bring together these aspects or to reformat previously developed standards to fit the umbrella idea of building biological systems.

Apart from this, the classical standardization requirements for science apply:

- i) Results need to be comparable between samples.
- ii) Results need to be comparable between labs.
- iii) Experiments need to be repeatable in several labs.
- iv) Researchers need to use the same language/naming.
- v) Data need to be amenable to electronic data processing.

5.2. The Biobricks Foundation, the RFC Process, iGEM, and the Registry of Standard Biological Parts

The most prominent efforts to organize and standardize Synthetic Biology are the formation of the Biobricks Foundation (BBF, <http://biobricks.org>), the “iGEM” competition (<http://www.igem.org>), and the “Registry of Standard Biological Parts” (<http://partsregistry.org>) (see Note 2). This concept evolves around the idea of developing and providing standardized genetics parts named “BioBricks,” which can be assembled in a more or less black box fashion to build more and more complex systems. In short:

1. The BioBricks Foundation is the legal owner of the “BioBricks”™ trademark.
2. The student competition iGEM educates future researchers in the use of BioBricks and is at the same time the main provider of BioBricks.
3. The Registry stores the BioBrick DNA and maintains a database with the description of the BioBricks.

There are many positive and interesting aspects of these initiatives, yet here the focus is on the standardization emerging from these efforts. After a few years with no formal process, the BioBricks

Foundation set up an RFC procedure following the style of the IETF RFCs described above (Subheading 4.3). This form of standardization is still in an early stage. Browsing the first set of BBF RFCs, one realizes that the proposed standardizations are mostly linked to issues arising within the iGEM competition. As BBF RFCs are not filtered before publishing, quality and relevance also vary significantly.

5.3. The Current Status of BioBrick Cloning

An ongoing discussion within the iGEM community dwells on what DNA assembly methods should or can be used. The current standard is an idempotent cloning strategy named BBF RFC 10 (20) placing each part between a defined prefix and suffix sequence, which each contains two restriction enzymes (EcoRI, XbaI and SpeI, PstI), which are consequently not allowed within the part.

Several modifications of the original standard (RFC 10) exist adapting it to fusion proteins (e.g., RFC 23, RFC 25) or alternative restriction enzymes (e.g., RFC 20, RFC 21). Since the iGEM competition pressures participating teams to adhere to these standards, a lot of effort is invested on making parts compatible with this standard. The seemingly simple issue of how to clone illuminates problems which plague standardization in molecular biology. Choices of RFC 10 are debated, for example, in RFC 12, RFC 20, RFC 21, RFC23, and RFC54. Everybody realizes that automation of a standard cloning method would significantly speed up the process and would have the potential to eliminate human errors. Unfortunately, a “one method fits all,” which is inexpensive, sequence independent, and scalable from small parts of a few base pairs to hundreds of kilo bases, remains elusive. Depending on the task, which can be cloning of fusion proteins requiring scar-free cloning, cloning of highly repetitive or self-complementary sequence stalling most known polymerase, or cloning of very large fragments and genome integration, tailored solutions are required, which at present are either performed manually or need individual adaption of a pipetting robot. In some labs, high-content cloning has been established for specific tasks, yet these methods often rely on expensive and/or extensive robots or proprietary vector systems and are thus hard to transfer between labs. In gene synthesis companies, the task of generating genes has reached a higher level of automation and prices dropped significantly to render many material transfer agreements unnecessary. Nonetheless, gene synthesis is not yet a replacement for efficient cloning. In summary, most labs would welcome speedier and more compatible cloning, but the job gets done and thus the activation barrier for a change is very high. Therefore, it requires a catalyst like iGEM to switch to a more common standard, even more so if the standard is far from being perfect and, from an individual standpoint, provides often only minor speed advantages.

5.3.1. Cloning According to BBF RFC 10 and RFC 25

The first BioBrick assembly standard was set before the development of the RFC process and is described in a working paper (21), which was then converted to RFC 10 (20). RFC 25 (22) is an extension of RFC 10 enabling the idempotent cloning of fusion proteins while maintaining full compatibility to RFC 10. The following protocol lists the RFC 10 and RFC 25 requirements for cloning BioBricks (Figs. 1 and 2).

RFC 10 Design Requirements

1. The BioBrick DNA sequence must not contain the restriction endonuclease sites: EcoRI GAATTC, XbaI TCTAGA, SpeI ACTAGT, PstI CTGCAG, NotI GCGGCCGC.
2. At the 5'-end, the BioBrick has to be flanked (see Notes 3–5):
 - (a) For non protein-coding Biobricks by the prefix 5'-GAATTC GCGGCCGC T TCTAGA G-3'
 - (b) For protein-coding Biobricks by the prefix 5'-GAATTC GCGGCCGC T TCTAG-3'; in this case, the start codon must be ATG
3. At the 3'-end, the BioBrick has to be flanked by the suffix 5'-T ACTAGT A GCGGCCG CTGCAG-3' (see Notes 6 and 7).

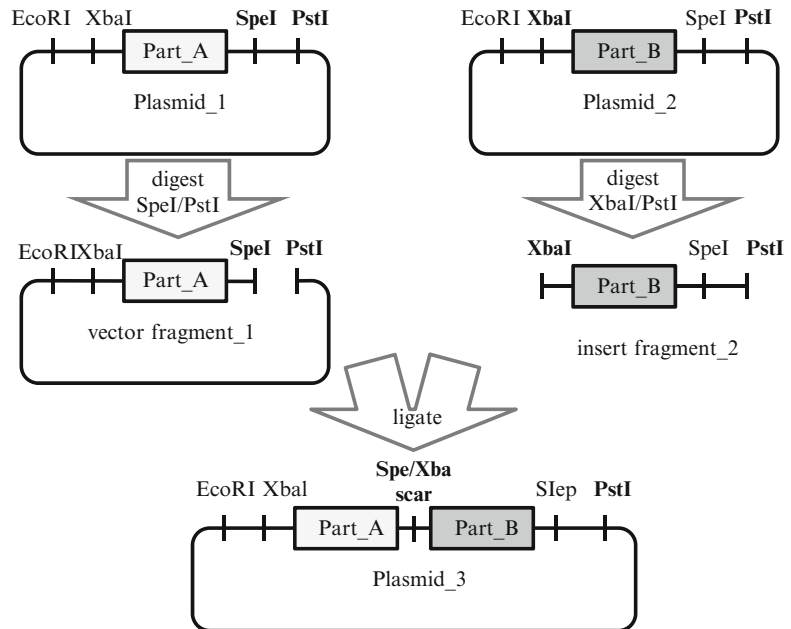


Fig. 1. Scheme of the RFC 10 idempotent cloning strategy. Plasmid_1 containing Part_A is digested with SpeI and PstI. Plasmid_2 containing Part_B is digested with XbaI and PstI. Vector fragment and insert are ligated and yield Plasmid_3 containing the composite part of A and B interspersed by the cloning scar and flanked by the standard prefix and suffix.



Fig. 2. Sequence of prefix and suffix according to RFC 25. The *underlined part* is identical to the RFC10 expression part definition. The additional restriction sites for NgoMIV and AgeI flank the part sequence, which is symbolized with “n.” Reading frame c of the shown sequence segment is given. The in-frame restriction sites code for AlaGly and ThrGly. Ligation of the compatible ends of AgeI and NgoMIV generates a scar coding for ThrGly.

4. BioBrick parts must be supplied in plasmids compatible with Registry and assembly procedures and may be pSB-series plasmids or other compliant plasmids.
5. It is recommended that the standard sequencing primers VF2: TGCCACCTGACGTCTAAGAA and VR: ATTACCGCCTTTGAGTGAGC work with the plasmid.
6. For PCR amplification of a gene with simultaneous addition of the prefix and suffix sequences, the following PCR primers are recommended: forward 5'-TCT TCG AAT TCG CGG CCG CTT CTA GAG AG-(18–24 bases of the gene)-3' and reverse 5'-GTT TCT TCC TGC AGC GGC CGC TAC TAG TA-(18–24 bases of the gene)-3'.

Cloning According
to RFC 10

The idea of RFC 10 is an idempotent strategy in which always the same restriction sites are used to clone into an existing BioBrick assembly. The scheme is illustrated in Fig. 1. The key feature is that XbaI and SpeI generate compatible ends, which upon ligation generate a cloning “scar” which cannot be cut by either enzyme.

For cloning BioBrick B after BioBrick A in a plasmid, follow this protocol:

1. Digest the plasmid with SpeI and PstI and isolate the vector fragment.
2. Digest the insert with XbaI and PstI, and isolate the insert fragment.
3. Mix insert with vector and ligate.
4. Transform in *E. coli* and plate on agar containing the appropriate antibiotic for the vector plasmid.

For cloning an assembly of BioBrick A-B-C-D, follow this protocol:

1. Assemble A-B and C-D according to the above protocol.
2. Clone C-D behind A-B following the above protocol.

RFC 25 Design
Requirements and Cloning
According to RFC 25

RFC 25 is an extension of RFC 10 utilizing the same idempotent strategy with two additional restriction enzymes located within the RFC 10 restriction sites and in frame with a coding sequence (Fig. 2). The two endonucleases used are NgoMIV and AgeI. The NgoMIV overhang (G'CCGG,C) is compatible with the AgeI overhang (A'CCGG,T) and ligation results in the scar ACCGCC coding for the two amino acids Thr and Gly, which are suited as linkers between protein domains.

The RFC 25 design requirements are the following:

1. Restriction sites for EcoRI, NotI, XbaI, NgoMIV, AgeI, SpeI, NotI, and PstI must not be present in the gene of interest.
2. At the 5' end of the gene of interest, the following prefix is required: gaattcgcggccgcttctagatggccggc.
3. At the 3'-end of the gene of interest, the following suffix is required: accggttaatactagtagcggccgctgcag.
4. If an N-terminal extension is not intended, e.g., if a protein fold does not permit N-terminal extension, an RFC10 expression part prefix can also be used without the NgoMIV addition.

Cloning of fusion proteins composed of protein domain A followed by domain B according to RFC 25 follows these steps:

1. Digest plasmid containing gene A with AgeI and PstI and isolate the vector fragment.
2. Digest plasmid containing gene B with NgoMIV and PstI and isolate the insert fragment.
3. Ligate the insert and vector fragment
4. Transform in *E. coli* and plate on agar containing the appropriate antibiotic for the vector plasmid.

5.4. Description of Parts

A statistic of 2009 published on the Registry Web site lists over 5,000 available BioBricks and states that almost 1,700 plasmids containing parts were sent to each team participating in iGEM. The Registry Web site provides access to the parts by type, e.g., promoter or protein coding; device type, e.g., reporter, inverter; or function, e.g., cell-cell signaling. The DNA sequence of a part and information entered by the users can be downloaded. As most parts are submitted by students under time pressure and up to iGEM 2010 the submission process of the part documentation and annotation was manual, the documentation quality of the parts varies significantly. An example of 116 part submissions from the authors' 2010 iGEM team can be found at http://partsregistry.org/Viral_vectors. The total number of parts in the Registry is impressive for a student competition, and it should not be compared to the number of clones offered by some companies which provide clones of each open reading frame from several organisms. The value of the Registry lies in

the fact that over thousands of students get trained in the use each year, the growth with each new student generation, and that over several years documentation and raking of parts evolve. Any lab can contribute to the Registry, although so far only few labs take this opportunity. A new development is the setup of the “Biofab” (<http://www.biofab.org>), which in the future might contribute parts in a larger scale.

The exact definition of a BioBrick and the handling of variations are not completely settled. At present, only DNA as plasmid is stored in the Registry. The BioBrick DNA sequence is given without the cloning prefix and suffix, since these depend on the cloning strategy chosen by the submitting party. Prefix and suffix are given as additional information. This is a very reasonable decision, but has in specific cases major consequences that are often overlooked, again pointing to problems once it comes to the details of standardization. Different choices of prefix and suffix can add cloning scars to protein-coding BioBricks, which are translated in amino acids. Different terminal ends of a protein can significantly influence translation, degradation (23), and stability (24) of proteins. As an undesired consequence, the description of a protein-coding part cannot be easily abstracted from the cloning strategy. A workaround would be to establish a subclassification at the level of single nucleotides. Together with the Registry, the authors’ iGEM 2010 team introduced such versioning for the standard Registry cloning vector, albeit only for mutations within the vector.

Once mutations of parts get versions, a typical biological question arises: Which degree of homology or identity justifies bundling in one part or separation in different parts? There is no objective rule to such a decision, and it is typically made based on the use in the community. For example, the fluorescent proteins GFP, YFP, Venus, CFP, and Cerulean got different names despite the high sequence identity because one important property is different, whereas most enzymes get the same name even at low identity level as long as they catalyze the same reaction. Mutations are inherent to biology and the research on alignments and annotations from protein domains to genomes is dealing with this. As functional tolerance to mutations is not yet fully understood, Synthetic Biology should provide part descriptions at the most detailed sequence level available. The need for an exact and easy accessible sequence for each experiment has resulted in the initiative for the essential information for synthetic DNA sequences (25).

Proposals of how a part description can look like have been published. Canto and colleagues define a standard part as “a genetically encoded object that performs a biological function and that has been engineered to meet specified design or performance requirements” (26). They provide an example of how the function of a device genetically composed of a Tet promoter, a ribosome-binding site, the luxR gene, a terminator, and lux-dependent

promoter and transformed into *E. coli* can be characterized. On one page, a prototypical “datasheet,” key measurements, and parameters of the input and output characteristics are listed. In addition, the authors provide the general setup of the experiment, such as the host strain, media, and temperature. The visual setup of a facts sheet is good for manually browsing parts; however, in the long run, a more detailed and computer readable format will prevail in case of complex systems. Several shortcomings of an attempt to describe a complex biological experiment with all possible variations can be listed, but the importance of an aggregated dataset should be emphasized and arbitrary but reasonable choices are a hallmark of many biological experiments and accepted as long as they serve the intended use (27).

5.4.1. Requirements for Parts Descriptions

The description of a BioBrick or any other component for Synthetic Biology is not settled and to some extent always depends on the intended use. As mentioned above, the listing of prefix and suffix sequences which potentially influence function is still debated. The following list, thus, can only serve as guideline:

1. Release of information:
 - (a) Information and data should be available in a widely accepted nonproprietary format on an open-access platform.
 - (b) To the widest extent possible, information should be made available in human- and machine-readable form.
 - (c) Information should be given in a short abstract and a full description.
 - (d) Information should be given in a standardized vocabulary if available.
 - (e) Information should be linked to the existing knowledge, such as databases.
2. Physical composition:
 - (a) History and methods of assembly.
 - (b) Complete DNA sequence (should be verified by sequencing).
 - (c) Annotation of the DNA sequence to the best-available knowledge, including features of the DNA, mRNA, and protein level, if applicable.
3. Experimental environment:
 - (a) Description of the host cell/chassis.
 - (b) Detailed description of relevant equipment and materials.
 - (c) Detailed description of the experimental parameters.
 - (d) Validation and calibration status of equipment.
 - (e) Contribution of each involved person.

4. Experimental results:
 - (a) Raw data if processing obscures deduction.
 - (b) Detailed information of how data are processed.
 - (c) Processed and aggregated data.
 - (d) Visualization of data.
5. Deduced function and parameters for higher abstraction layers:
 - (a) Short description of intended implementation.
 - (b) The exact amount of parameters required to implement and control the part.
 - (c) Working range of the parameters.
 - (d) Estimate of robustness with respect to parameter constellation and time.
6. Security and safety:
 - (a) Known and anticipated security issues.
 - (b) Known and anticipated safety issues.
7. Legal:
 - (a) If available, information on the patent situation.
 - (b) Any intellectual property claims made by the authors.
 - (c) Conflict of interest by the authors.

6. Language and Data Processing Standards in Biological Sciences

A very important aspect for enabling large-scale data integration is the development of a common, standardized language. Data interoperability is achieved using formal representations of facts as well as concepts, linking and processing the data. These ontologies are widespread in biological sciences. As an umbrella organization to coordinate ontologies development, the Open Biological and Biomedical Ontologies foundry (<http://www.obofoundry.org/>) was formed (28). One of the most prominent ontologies is the gene ontology (GO, <http://www.geneontology.org/>) (29). This ontology “covers three domains: (1) *cellular component*, the parts of a cell or its extracellular environment; (2) *molecular function*, the elemental activities of a gene product at the molecular level, such as binding or catalysis; and (3) *biological process*, operations or sets of molecular events with a defined beginning and end, pertinent to the functioning of integrated living units: cells, tissues, organs, and organisms.”

At present, many ontologies are encoded following the Web Ontology Language (OWL, <http://www.w3.org/TR/owl-ref>) based on the Resource Description Framework (RDF, <http://www.w3.org/TR/rdf-concepts/>) and using the Extensible Markup

Language (XML, <http://www.w3.org/TR/xml/>) serialization. These semantic Web standards have been developed by the World Wide Web Consortium (W3C, <http://www.w3.org/>) using their standardization process (<http://www.w3.org/standards/about.html>).

Next to genetics, formal data handling and ontologies are also widespread in systems biology. The System Biology Markup Language (SBML, <http://sbml.org>) (30) defines an interchange format for computer models of biological processes. The Biopax project (<http://www.biopax.org>) (31) aims to enable integration, exchange, visualization, and analysis of biological pathway data. An overview of various standards in systems biology is given in (32). The link between systems and Synthetic Biology is evident since both disciplines require an abstracted description of biological processes (33). Currently, there are over 150 software packages supporting SBML, which are also useful for Synthetic Biology (34) and can be used for computational design and engineering of biological parts (34, 35).

Specific for Synthetic Biology is the development of the Synthetic Biology Open Language (SBOL, <http://www.sbolstandard.org/>). A proposal for the use of RDF in the frame of Synthetic Biology has been published as a BBF RFC 30 (36) and BioBrick information has been made available in SBOL (37).

7. Legal Considerations

For international DNA repositories collecting and distributing community-supplied material, the legal framework is of outmost importance (38). The patent situation in biotechnology is hard to oversee. Many researchers, particularly in academic settings, are not sufficiently aware of the patent situation and do not have access to intellectual property specialists. The BioBricks foundation has set up a draft contributor agreement for submitting parts to the Registry (39).

8. Notes

1. For certain procedures (for example, sampling in a toxicokinetic study), very exact timing is necessary and the data must demonstrate that the schedule was strictly followed.
2. All three initiatives have mainly been developed by a group of people colocated at the MIT at the time of the conceptual layout.

3. For protein-coding RFC 10 BioBricks, the A nucleotide of the start codon becomes part of the XbaI restriction site.
4. The additional T between NotI and XbaI site is inserted to prevent inadvertent creation of EcoBI or EcoKI methylation sites (21); EcoKI methylase modifies adenine residues in the sequences AAC(N6)GTGC and GCAC(N6)GTT.
5. The additional G after the XbaI site is inserted to prevent creation of a Dam methylation site; Dam methylates the N⁶-position of the adenine in the sequence GATC. Thus, an XbaI site followed by TC could be blocked.
6. If constructing a primer, this sequence must be reverse complemented.
7. The T preceding the SpeI is a required base.

References

1. Leduc, S. (1912) La biologie synthétique, étude de biophysique (Poinat, A., Ed.). Paris.
2. Szybalski, W., and Skalka, A. (1978) Nobel prizes and restriction enzymes., *Gene* 4, 181–2.
3. Gaiser, S., Reiss, T., Lunkes, A., Müller, K. M., and Bernauer, H. (2009) Making the most of synthetic biology. Strategies for synthetic biology development in Europe., *EMBO reports* 10 Suppl 1, S5–8.
4. Berman, H. M. (2008) The Protein Data Bank: a historical perspective., *Acta crystallographica. Section A, Foundations of crystallography*. International Union of Crystallography 64, 88–95.
5. Strasser, B. J. (2008) Genetics. GenBank – Natural history in the 21st Century?, *Science (New York, N.Y.)* 322, 537–8.
6. Quackenbush, J. (2009) Data reporting standards: making the things we use better., *Genome medicine* 1, 111.
7. Kettner, C., Field, D., Sansone, S.-A., Taylor, C., Aerts, J., Binns, N., Blake, A., Britten, C. M., Marco, A. de, Fostel, J., Gaudet, P., González-Beltrán, A., Hardy, N., Hellemans, J., Hermjakob, H., Juty, N., Leebens-Mack, J., Maguire, E., Neumann, S., Orchard, S., Parkinson, H., Piel, W., Ranganathan, S., Rocca-Serra, P., Santarsiero, A., Shotton, D., Sterk, P., Untergasser, A., and Whetzel, P. L. (2010) Meeting Report from the Second “Minimum Information for Biological and Biomedical Investigations” (MIBBI) workshop., *Standards in genomic sciences* 3, 259–66.
8. Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J., Ansorge, W., Ball, C. A., Causton, H. C., Gaasterland, T., Glenisson, P., Holstege, F. C., Kim, I. F., Markowitz, V., Matese, J. C., Parkinson, H., Robinson, A., Sarkans, U., Schulze-Kremer, S., Stewart, J., Taylor, R., Vilo, J., and Vingron, M. (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data., *Nature genetics* 29, 365–71.
9. Taylor, C. F., Paton, N. W., Lilley, K. S., Binz, P., Jalain, Jr, R. K. J., R, A., Zhu, W., Apweiler, R., Aebersold, R., Deutsch, E. W., Dunn, M. J., Heck, A. J. R., Leitner, A., Macht, M., Mann, M., Martens, L., Neubert, T. A., Patterson, S. D., Ping, P., Seymour, S. L., Souda, P., Tsugita, A., Vandekerckhove, J., and Vondriska, T. M. (2007) The minimum information about a proteomics experiment (MIAPE), *Nature Biotechnology* 25, 887–893.
10. Vizcaíno, J. A., Martens, L., Hermjakob, H., Julian, R. K., and Paton, N. W. (2007) The PSI formal document process and its implementation on the PSI website., *Proteomics* 7, 2355–7.
11. Gibson, F., Anderson, L., Babnigg, G., Baker, M., Berth, M., Binz, P.-A., Borthwick, A., Cash, P., Day, B. W., Friedman, D. B., Garland, D., Gutstein, H. B., Hoogland, C., Jones, N. A., Khan, A., Klose, J., Lamond, A. I., Lemkin, P. F., Lilley, K. S., Minden, J., Morris, N. J., Paton, N. W., Pisano, M. R., Prime, J. E., Rabilloud, T., Stead, D. A., Taylor, C. F., Voshol, H., Wipat, A., and Jones, A. R. (2008) Guidelines for reporting the use of gel electrophoresis in proteomics., *Nature biotechnology* 26, 863–4.
12. Orchard, S., Salwinski, L., Kerrien, S., Montecchi-Palazzi, L., Oesterheld, M., Stümpflen, V., Ceol, A., Chatr-aryamontri, A., Armstrong, J., Woollard, P., Salama, J. J.,

- Moore, S., Wojcik, J., Bader, G. D., Vidal, M., Cusick, M. E., Gerstein, M., Gavin, A.-C., Superti-Furga, G., Greenblatt, J., Bader, J., Uetz, P., Tyers, M., Legrain, P., Fields, S., Mulder, N., Gilson, M., Niepmann, M., Burgoon, L., De Las Rivas, J., Prieto, C., Perreau, V. M., Hogue, C., Mewes, H.-W., Apweiler, R., Xenarios, I., Eisenberg, D., Cesareni, G., and Hermjakob, H. (2007) The minimum information required for reporting a molecular interaction experiment (MIMIX)., *Nature biotechnology* 25, 894–8.
13. Taylor, C. F., Binz, P.-A., Aebersold, R., Affolter, M., Barkovich, R., Deutsch, E. W., Horn, D. M., Hühner, A., Kussmann, M., Lilley, K., Macht, M., Mann, M., Müller, D., Neubert, T. A., Nickson, J., Patterson, S. D., Raso, R., Resing, K., Seymour, S. L., Tsugita, A., Xenarios, I., Zeng, R., and Julian, R. K. (2008) Guidelines for reporting the use of mass spectrometry in proteomics., *Nature biotechnology* 26, 860–1.
14. Jones, A. R., Carroll, K., Knight, D., MacLellan, K., Domann, P. J., Legido-Quigley, C., Huang, L., Smallshaw, L., Mirzaei, H., Shofstahl, J., and Paton, N. W. (2010) Guidelines for reporting the use of column chromatography in proteomics, *Nature Biotechnology*. Nature Publishing Group 28, 654–654.
15. Hoogland, C., O Gorman, M., Bogard, P., Gibson, F., Berth, M., Cockell, S. J., Ekefjård, A., Forsstrom-Olsson, O., Kapferer, A., Nilsson, M., Martínez-Bartolomé, S., Albar, J. P., Echevarría-Zomeño, S., Martínez-Gomariz, M., Joets, J., Binz, P.-A., Taylor, C. F., Dowsey, A., and Jones, A. R. (2010) Guidelines for reporting the use of gel image informatics in proteomics, *Nature Biotechnology*. Nature Publishing Group 28, 655–656.
16. Domann, P. J., Akashi, S., Barbas, C., Huang, L., Lau, W., Legido-Quigley, C., McClean, S., Neusüß, C., Perrett, D., Quaglia, M., Rapp, E., Smallshaw, L., Smith, N. W., Smyth, W. F., and Taylor, C. F. (2010) Guidelines for reporting the use of capillary electrophoresis in proteomics, *Nature Biotechnology* 28, 654–655.
17. WHO. (2009) HANDBOOK GOOD LABORATORY PRACTICE (GLP) (Kioy, D., Long, D., Bhalla, S., and Seiler, J., Eds.).
18. Schulz, K. F., Altman, D. G., and Moher, D. (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials., *BMJ (Clinical research ed.)* 340, c332.
19. Andrianantoandro, E., Basu, S., Karig, D. K., and Weiss, R. (2006) Synthetic biology: new engineering rules for an emerging discipline, *Molecular Systems Biology* 1–14.
20. Knight, T. (2007) BBF RFC 10: Draft Standard for Biobrick Biological Parts, <http://dspace.mit.edu/handle/1721.1/45138>.
21. Knight, T. (2003) Idempotent Vector Design for Standard Assembly of Biobricks, <http://dspace.mit.edu/handle/1721.1/21168> 1–11.
22. Müller, K. M., Arndt, K. M., IGEM_Freiburg, 2007, and Grünberg, R. (2008) BBF RFC 25: Fusion Protein (Freiburg) BioBrick assembly standard, <http://dspace.mit.edu/handle/1721.1/45140>.
23. Hwang, C.-S., Shemorry, A., Auerbach, D., and Varshavsky, A. (2010) The N-end rule pathway is mediated by a complex of the RING-type Ubr1 and HECT-type Ufd4 ubiquitin ligases., *Nature cell biology*. Nature Publishing Group 12, 1177–85.
24. Hecky, J., and Müller, K. M. (2005) Structural perturbation and compensation by directed evolution at physiological temperature leads to thermostabilization of beta-lactamase., *Biochemistry* 44, 12640–54.
25. Peccoud, J., Anderson, J. C., Chandran, D., Densmore, D., Galdzicki, M., Lux, M. W., Rodriguez, C. A., Stan, G.-bart, and Sauro, H. M. (2011) Essential information for synthetic DNA sequences., *Nature biotechnology*. Nature Publishing Group 29, 22; discussion 22–3.
26. Canton, B., Labno, A., and Endy, D. (2008) Refinement and standardization of synthetic biological parts and devices., *Nature Biotechnology* 26, 787–93.
27. Arkin, A. (2008) Setting the standard in synthetic biology., *Nature biotechnology* 26, 771–4.
28. Smith, B., Ashburner, M., Rosse, C., Bard, J., Bug, W., Ceusters, W., Goldberg, L. J., Eilbeck, K., Ireland, A., Mungall, C. J., Leontis, N., Rocca-Serra, P., Ruttenberg, A., Sansone, S.-A., Scheuermann, R. H., Shah, N., Whetzel, P. L., and Lewis, S. (2007) The OBO Foundry: coordinated evolution of ontologies to support biomedical data integration., *Nature biotechnology* 25, 1251–5.
29. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium., *Nature genetics* 25, 25–9.
30. Hucka, M., Finney, A., Sauro, H. M., Bolouri, H., Doyle, J. C., Kitano, H., Arkin, A. P., Bornstein, B. J., Bray, D., Cornish-Bowden, A., Cuellar, A. A., Dronov, S., Gilles, E. D., Ginkel, M., Gor, V., Goryanin, I. I., Hedley, W.

- J., Hodgman, T. C., Hofmeyr, J.-H., Hunter, P. J., Juty, N. S., Kasberger, J. L., Kremling, A., Kummer, U., Le Novère, N., Loew, L. M., Lucio, D., Mendes, P., Minch, E., Mjolsness, E. D., Nakayama, Y., Nelson, M. R., Nielsen, P. F., Sakurada, T., Schaff, J. C., Shapiro, B. E., Shimizu, T. S., Spence, H. D., Stelling, J., Takahashi, K., Tomita, M., Wagner, J., and Wang, J. (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models., *Bioinformatics (Oxford, England)* 19, 524–31.
31. Demir, E., Cary, M. P., Paley, S., Fukuda, K., Lemer, C., Vastrik, I., Wu, G., D Eustachio, P., Schaefer, C., Luciano, J., Schacherer, F., Martinez-Flores, I., Hu, Z., Jimenez-Jacinto, V., Joshi-Tope, G., Kandasamy, K., Lopez-Fuentes, A. C., Mi, H., Pichler, E., Rodchenkov, I., Splendiani, A., Tkachev, S., Zucker, J., Gopinath, G., Rajasimha, H., Ramakrishnan, R., Shah, I., Syed, M., Anwar, N., Babur, Ö., Blinov, M., Brauner, E., Corwin, D., Donaldson, S., Gibbons, F., Goldberg, R., Hornbeck, P., Luna, A., Murray-Rust, P., Neumann, E., Reubenacker, O., Samwald, M., Iersel, M. van, Wimalaratne, S., Allen, K., Braun, B., Whirl-Carrillo, M., Cheung, K.-H., Dahlquist, K., Finney, A., Gillespie, M., Glass, E., Gong, L., Haw, R., Honig, M., Hubaut, O., Kane, D., Krupa, S., Kutmon, M., Leonard, J., Marks, D., Merberg, D., Petri, V., Pico, A., Ravenscroft, D., Ren, L., Shah, N., Sunshine, M., Tang, R., Whaley, R., Letovksy, S., Buetow, K. H., Rzhetsky, A., Schachter, V., Sobral, B. S., Dogrusoz, U., McWeeney, S., Aladjem, M., Birney, E., Collado-Vides, J., Goto, S., Hucka, M., Novère, N. L., Maltsev, N., Pandey, A., Thomas, P., Wingender, E., Karp, P. D., Sander, C., and Bader, G. D. (2010) The BioPAX community standard for pathway data sharing, *Nature Biotechnology* 28, 935–942.
32. Strömbäck, L., Hall, D., and Lambrix, P. (2007) A review of standards for data exchange within systems biology., *Proteomics* 7, 857–67.
33. Church, G. M. (2005) From systems biology to synthetic biology., *Molecular systems biology* 1, 2005.0032.
34. Macdonald, J. T., Barnes, C., Kitney, R. I., Freemont, P. S., and Stan, G.-B. V. (2011) Computational design approaches and tools for synthetic biology., *Integrative biology : quantitative biosciences from nano to macro* 97–108.
35. Matsuoka, Y., Ghosh, S., and Kitano, H. (2009) Consistent design schematics for biological systems: standardization of representation in biological engineering., *Journal of the Royal Society, Interface/the Royal Society* 6 Suppl 4, S393-404.
36. Grünberg, R. (2009) BBF RFC 30: Draft of an RDF-based framework for the exchange and integration of Synthetic Biology data Relation to other BBF RFCs, <http://hdl.handle.net/1721.1/45143> 1–13.
37. Galdzicki, M., Rodriguez, C., Chandran, D., Sauro, H. M., and Gennari, J. H. (2011) Standard Biological Parts Knowledgebase, *PLoS ONE* (Schönbach, C., Ed.) 6, e17005.
38. Rai, A., and Boyle, J. (2007) Synthetic biology: caught between property rights, the public domain, and the commons., *PLoS biology* 5, e58.
39. Endy, D., and Grewal, D. (2010) The BioBrick Public Agreement DRAFT Version 1a, <http://dspace.mit.edu/handle/1721.1/50999> 1–8.

Synthetic Gene Networks

Methods and Protocols

Weber, W.; Fussenegger, M. (Eds.)

2012, XI, 393 p. 67 illus., 3 illus. in color., Hardcover

ISBN: 978-1-61779-411-7

A product of Humana Press