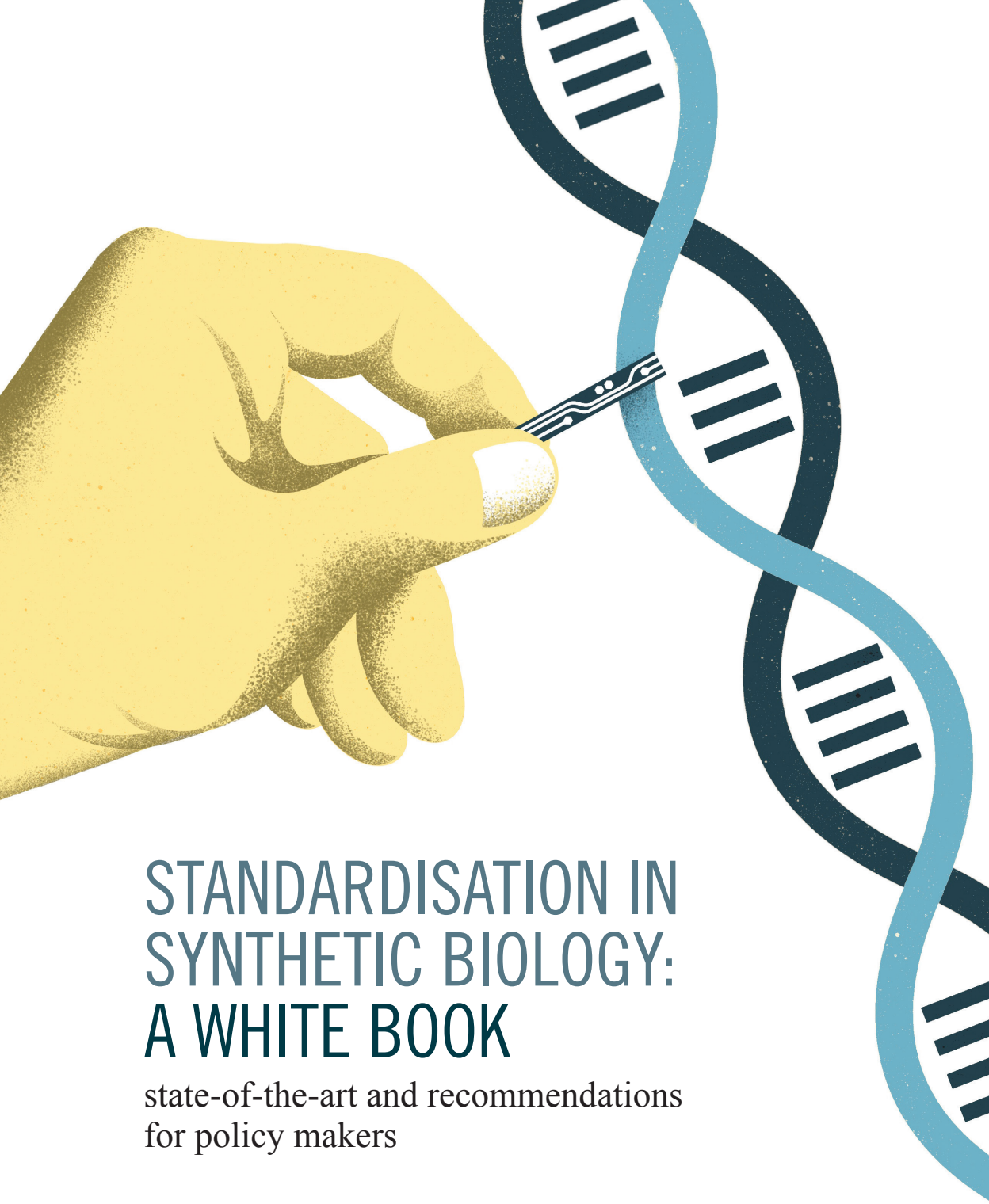

STANDARDISATION IN SYNTHETIC BIOLOGY: A WHITE BOOK

state-of-the-art and recommendations for policy makers

WRITTEN BY:

Elena Ordozgoiti, Manuel Porcar, Geoff Baldwin, Víctor de Lorenzo, Leonardo Ríos, Alistair Elfick, Pablo Schyfter, Ana Maria Delgado, Markus Schmidt, Michele Garfinkel and Lei Pei.

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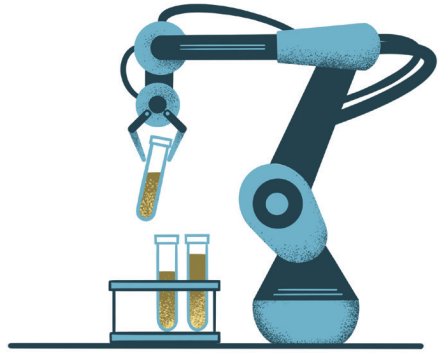
ABBREVIATIONS

Abs_{600}: absorbance at 600 nm
CD: Central Dogma
CEN: European Committee for Standardization
CENELEC: European Committee for Electrotechnical Standardization
CoC: Certainty of Containment
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
DICOM-SB: Digital Imaging and Communications in Medicine – Synthetic Biology
ERA: Environmental Risk Assessment
ETSI: European Telecommunications Standards Institute
EFSA: European Food Safety Authority
EMA: European Medicines Agency
GMO: Genetically Modified Organism
GCP: Good Clinical Practice
GLP: Good Laboratory Practice
GMP: Good Manufacturing Practice
GFP: Green Fluorescent Protein
HGT: Horizontal Gene Transfer
IEC: International Electrotechnical Commission
IP: Intellectual Property
iGEM: international Genetically Engineered Machine
ISO: International Organisation for Standardisation
ITU: International Telecommunication Union
JIMB: Joint Institute for Metrology in Biology
JBEI-ICEs: Joint BioEnergy Institute Inventory of Composable Elements
KBBE: Knowledge-Based Bio-Economy
MSB: Mammalian Synthetic Biology
NIST: National Institute of Standards and Technology
OSs: Operating Systems
PoPs: Polymerase Per Second
rDNA: recombinant DNA
RRI: Responsible Research & Innovation
RT-PCR: reverse-transcriptase PCR
RiPs: Ribosome Per Second
RNASeq: RNA sequencing
SbD: Safety by Design
SB: Synthetic Biology
SBA: Synthetic Biology Agent
SBML: Systems Biology Markup Language
SBOL: Synthetic Biology Open Language
SCENIHR: Scientific Committee for Emerging and Newly Identified Health Risks
SI: System Internationale
SOP: Standard Operating Procedures
TC: Technical Committee
FDA: Food and Drug Administration
YBA: Yeast BioBricks Assembly

”(…) BUT THE ONE THAT IS MOST ADAPTABLE TO CHANGE”

CHARLES DARWIN

PART 1
INTRODUCTION



C1_
STANDARDS AND THE INDUSTRY

"Standard" is a commonly used word whose first known use dates back to the 12th century, with different meanings throughout history and contexts. Companies, industrial consortia, non-governmental organisations and other entities develop standards with different uses and purposes and with different support behind them.

In this context, reference is made to technical documents known as standards which include characteristics, specifications, requirements or guidance that materials, products, services and processes shall comply with to ensure they are fit for purpose. These documents result from a common effort among experts, that work together to build consensus on a specific topic and for a particular purpose. They are publicly available and of voluntary application, and everyone can use and consult them. These documents are published by a network of standardisation bodies at international, European and national level, whose main function is to prepare, approve or adopt standards, and that work in a coordinated and cooperative manner to avoid duplication of efforts.

In an interconnected and continuously changing world as the one we are living in, although largely invisible, standards provide safety and security, compatibility and rationality to the value chain. Without standards, the society as we know it would not be possible, and professionals and regulators are well aware of this.

Standards are simply agreements between cooperating parts, and provide a common understanding for a given activity or process by facilitating production, cooperation and marketing, while boosting the economy as a whole. As they provide solutions for repeated or new situations, they improve efficiency, facilitate the exchange of information, limit undesirable failures and also reduce unbalanced asymmetry between parts. Frequently, standards support the implementation of public policies by developing the technical solutions that companies require to be able to comply with the principles established by the different regulations. Laboratories, companies and regulatory authorities can trust the contents of standards to use them in their regular business and regulatory acts.

Far from the idea that standards limit innovation and creativity, standardisation adds value to research, development and innovation projects and activities. They all benefit when they are built upon the knowledge and information that is included into internationally recognised standards, but also when part of the results and developments are included into new standards or are used to revise existing standards that will be updated with new, more accurate information.

Standards are a powerful tool for the dissemination of the findings and innovative solutions resulting from research, development and innovation projects. The inclusion of these findings and solutions into standards increases their dissemination as reliable information that has been approved and accepted by many experts in the field at international level. Standards are therefore key to increase their acceptability and potential access to market. In other words, they are key to maximise the economic and social impact of innovation.

The standardisation system consists of a network of organisations ruled by similar principles and objectives: the preparation of standards through a transparent and consensus building process. The final documents will be made available to the public in

the official language of the publishing organisation but can also be translated into other languages by national member bodies, which multiplies their understanding and use.

Standardisation bodies at European level (CEN and CENELEC) and at international level (ISO and IEC) work under the national delegation principle registering only one national organisation per country, which must be recognised by its government. Participation in the European Telecommunications Standards Institute (ETSI) is directly open for companies, and the International Telecommunication Union (ITU) is composed of the governments from the different countries. National standardisation bodies are open to the participation of all interested parties in a transparent and cooperative process and will convey the national position to the corresponding European or international organisation.

European and international standardisation bodies, such as CEN, CENELEC, ISO and IEC, develop and publish several types of documents, with the Standard being the document that represents the highest level of consensus and the broadest possible consultation. Other types of documents include ones that adapt to the market requirements, such as those of fast developing technologies or incipient sciences whose methods and models have not yet been proved and need a period of experimental work to test their efficacy. Their inclusion in a document published by an international member of the standardisation system gives them the worldwide diffusion they need to be tried out.

Between the 6 existing international organisations, all areas of knowledge and economic activity are covered, including electrical and electronics, telecommunications, and many others. These are some examples of the types of documents they develop and the fields they tackle:

- Quality management standards to help work more efficiently and reduce product failures.
- Environmental management standards to help reduce environmental impacts, reduce waste and be more sustainable.

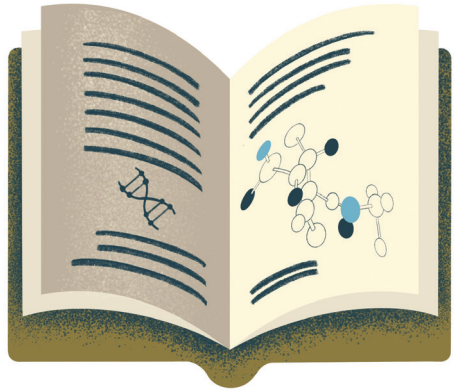
– Health and safety standards to help reduce accidents in the workplace.
– Energy management standards to help reduce energy consumption.
– Food safety standards to help prevent food from being contaminated.
– IT security standards to help keep sensitive information secure.

In the field of biotechnology, there is an international Technical Committee (TC), ISO/TC 276 "Biotechnology" which involves the participation of 48 countries. This TC has already published 15 standards and has another 20 under development on topics such as biobanking, bioprocessing, cell counting or data publication.

Furthermore, attention must be paid not only to the above-mentioned standards, but also to community standards. There is a significant number of organisations that have established community standards (SBOL, SBML, GA4GH, among others) with a special focus on specific areas or topics. These will be further explored in subsequent chapters of the present whitebook.

Conclusion

Standardisation is a complex, hierarchical process which can be applied to any engineering field. Paradoxically enough, Synthetic Biology (SB), the scientific field aiming at "making life easier to engineer" is still missing a massive development -not to say adoption- of biological standards. The present white book addresses the reasons, challenges and suggestions to tackle the exciting challenge of standardising biology.



C2_
STANDARDS IN BIOLOGY: STATE OF THE ART

Why standards? [1]

The onset of Systems and Synthetic Biology and the emphasis that these disciplines place on rigorous quantification and description of biological objects in their whole multi-scale complexity has raised the opportunity to look at live entities through an authentic (not just metaphoric) engineering perspective. This view stresses the cataloguing of the systems' components, the relational logic that allows them to work as they do and the definition of the boundaries between the different organisational levels and modules. Within this framework, the agenda of the modern Biotechnology that builds on Systems and Synthetic Biology is to make the design of live objects an authentic engineering discipline. This requires bringing the issue of standards to the biological realm, a matter that has been generally alien to Life Sciences research. It is incorrectly assumed that standards sacrifice flexibility and limit the freedom to operate. Standards provide enormous advantages in efficiency and reproducibility.

When different communities wish to work together they need to adopt standards that enable their interplay in time and space (Table 1). Standards allow decoupling design from production from assembly from deployment, and they help to reduce the lack of reproducibility of results that plague the scientific and technical liter-

1 This section is an abridged and updated version of de Lorenzo & Schmidt (2018).

ature in Biology and Biotechnology. But what can be the subject of standardisation efforts at this time in the biotechnological domain? The majority of the attempts to tackle this issue have focused thus far on microorganisms. They are the biological systems of immediate biotechnological value and the most amenable to deep genetic engineering with the currently available technologies. Note, however, that bacteria are being rapidly caught up by yeast and plants as biological *chassis* amenable to sound bio-programming. Furthermore, the scope of SB also includes cells-free systems, which can be subjected to standardisation but lack (at least for now) the scalability and self-replication properties of whole cells that make living microorganisms so appealing for industrial applications.

Table 1. What is involved in standardisation

Standardisation subject	Standardisation challenge
Physical assembly of system components	Definition of geometrical shapes
	Specification of dimensions
	Compatibility of boundaries between elements
	Compositional rules
Metrology	Units of measurement of relevant properties
	Conditions and procedures to calculate units
	Reference values and objects
	Tolerance and allowance
	Context sensitivity
	Transfer functions
Handling/manufacture of engineered objects	Standard operating procedures (SOPs)
	ISO standards

Formal languages	System description
	Workflow description
	Data exchange
	Programming (operative systems)
Databases	Spread sheets / work sheets
	Metadata
	Interoperability/Compatibility
Risk assessment	Safety criteria
	Security benchmarks
Ethical appraisal	Consensus rules
Promulgation	Enforcement
Intellectual property management	Patents, Open Access, Open Source

Standards for tackling the gene expression flow

From a SB perspective, there are two major aspects to address when engineering live systems. One is the compositional layout, which is traditionally abstracted as layers of growing complexity from *parts* to *devices* to *systems*, with a possible intermediate stage of *modules*. The second feature is the flow of information through the system, which coincides with the Central Dogma (CD) of Molecular Biology: *DNA* to *RNA* to proteins—and from there, to specific functionalities, biochemistry, or other aspects (Figure 1). That the material architecture (and thus the compositional logic) of any live system is itself derived from the gene expression flow places most standardisation efforts in the different phases of such a process. As every textbook would say, a cod-

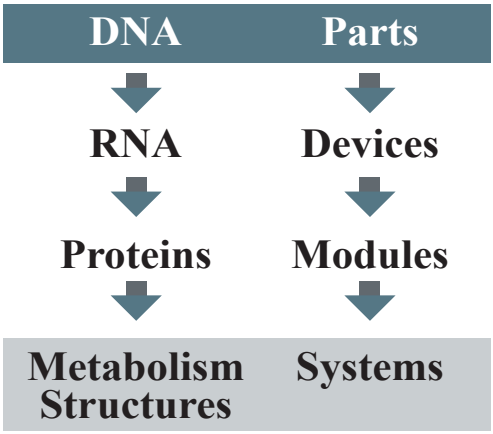


Figure 1. The Central Dogma of Molecular Biology *vs* the abstraction hierarchy of Bioengineering. While the Central Dogma exposes the transfer of information through the gene expression flow, the conceptual framework of Synthetic Biology allows the assembly of complex biological systems on the basis of rationally composing parts and devices. Although the two schemes follow entirely different roadmaps, they intersect at the beginning (parts-DNA) and the end (systems-metabolism/structures). Source: de Lorenzo & Schmidt (2018).

ing DNA sequence can be *transcribed* to produce mRNA, which is in turn *translated* to give functional proteins. The qualitative picture is straightforward but altogether useless for robust engineering unless it is endowed of quantitative parameters, transfer functions, error detection and correction and context-dependency data. Thus, developing standards for these aspects is badly needed in order to make progress in real bioengineering.

Physical vs functional composition of biological systems

DNA is ultimately a physical object and, as such, can be manipulated to join other DNA segments. It comes as no surprise that the last few years have witnessed the booming of a large number of stratagems for the standard assembly (i.e. physically composing) of increasingly large sequences. Yet, the immediate question in this regard is how *physical* composition becomes *functional* composition i.e. whether parts can be reused maintaining their original properties and associated parameters. The experience of the Biological and Biotechnological communities indicate that the assembly of DNA parts often results in genetic devices that may function as expected from the qualitative point of view, but not quantitatively. Genomic and biochemical context sensitivity (including physical location of the genes or the products in given locations of the 3D structure of the cell) and

environmental conditions may altogether change the functioning of the parts and devices of interest. In addition, designed biological systems often develop emergent properties in which the readout of the pursued phenotype may be more or less than the mere sum of its parts. This is often influenced by the small molecules that abound in any biological milieu. Last but not least, biological systems are subject to Darwinian evolution, which seems to quickly erase or silence human-made changes that cause a decrease of fitness.

It is true that one can agree to very specific conditions that enable inter-laboratory reproducibility studies. But the same tests highlight how context dependent biological components are and how easily they may vary even with anecdotal environmental changes. The ultimate way out from this situation relies on acquiring more fundamental knowledge on the rules that govern the appearance of distinct functionalities in extant biological systems through the gene flow from DNA to proteins in time and space—an issue that has received considerable attention in recent times. But what to do in the meantime? The development of improved vectors and DNA assembly strategies that mitigate the problem of physical *vs.* functional composition will still be necessary for several years, in particular for engineering or streamlining the genomic complement of non-model bacteria, of which less fundamental knowledge is available. One contribution in this direction was the launch in 2013 of the Standard European Vector Architecture (SEVA; http://seva.cnb.csic.es), a repository of formatted molecular tools for de-constructing and re-constructing complex prokaryotic phenotypes beyond *Escherichia coli.* The SEVA is currently helping to fill the phenomenal gap between the existing DNA synthesis tools and the actual engineering of predictable and efficacious bacteria. Yet, although this gap is bound to rapidly become narrow, the question still remains on how to convert the physical composition of DNA segments encoding genes and signals into a predictable and stable performance of the cognate bio-engineered live objects.

Metrology

Besides the challenge of standardising assembly rules (and quite intertwined with it), the second big question of Bioengineering deals with accurately measuring biological activities. While the compositional challenge of creating multi-scale biological complexity as a progression from parts to devices to modules to systems is well defined (see above), the establishment of standards for describing, measuring and rewiring key biological functionalities (as well as suitable platforms and languages for data exchange) is still a bottleneck. What is needed is the development of new types of technologies that could be called *in vivo Biomolecular Metrology*. This is not only about proposing unequivocal units to describe the activity at stake but also to figure out objects of reference for calibration so as to enable the coordination of measurements across distant locations and over time. The first steps to develop a robust biological metrology starts with addressing transcription and translation.

The idea of having a universal measure for transcriptional activity of given promoters was already present at the foundation of SB as a biological counterpart of electric current. The term PoPs (Polymerase Per Second) was coined to describe the number of times RNA polymerases pass a promoter sequence to originate a productive transcript. Although transcription initiation and quality of the resulting mRNA are in themselves quite complex and densely regulated biological events, it is possible to make a first approximation to gene expression activity by adopting such PoPs units. The next obvious question is how to measure them. Although available procedures to this end are fastidious and time-consuming (and still context-dependent, see above), having a set of well-calibrated promoters in terms of their actual PoPs could be a phenomenal step for biological metrology—nor unlike the definition of Amperes in electricity. This could also pave the way for defining biological counterparts of Ohms (i.e. anything that impedes the progress of RNA polymerase from one promoter to the next one) and Volts (i.e. inherent promoter strength).

The second step in the gene expression flow is translation, which could also be abstracted and parameterised as RiPs (Ribosome Per Second), which refers to the number of ribosomes that pass productively through an mRNA sequence to deliver a full-length protein. Although the abstract concept is clear, the mechanisms involved in the process are extremely intricate, in particular the control of mRNA stability and the possible targeting of mRNAs to different sites of the cell. Ribosome profiling could help to determine such RiPs parameters, but development of simpler techniques to the same end could be envisioned, with the same possible dividends as discussed above for PoPS.

Languages for engineering Biology

A third standardisation front deals with languages—both for [a] description and exchange of biological data and phenomena as for [b] programming cells with new capabilities. The first aspect has already received considerable attention in the realm of Systems Biology and various propositions on the matter have been entertained over the years. One of the simplest involves logic gates: regulatory networks possess a large number of control modules that formally implement many of the operations that are typical of digital, Boolean circuits. As the corresponding biological transactions adopt somewhat continuous values, the 0/1 states are generally agreed to reflect low/high states for the input status and off/on for output promoter activity. Logic gates based on promoters and transcriptional factors provide an attractive and simple (while also scalable) framework for both describing and designing artificial biological circuits, as a virtually unlimited diversity of schemes can be produced by just combining a relatively small number of modules. Far more sophisticated approaches, in the form of community standards, include the Systems Biology Markup Language (SBML, http://sbml.org) and the Synthetic Biology Open Language (SBOL http://sbolstandard.org). The latter focuses on genetic designs through a standardised vocabulary of schematic glyphs as well as a standardised digital format. One major appeal of SBOL is the specification of unequivocal rules

to visually represent either natural or engineered genetic circuits, which can then be enriched, also following strict rules, with experimental and computational data. This allows detailed descriptions not only of specific circuits, but also of entire workflows of biological engineering. The SBOL format is rapidly being adopted by a large number of communities (including journals) and it may end up being the preferred instrument of communication between biological systems, human users, computational resources and even robotic platforms for remote experimentation.

There is still another type of standard languages: those that allow programming cells to sense signals, run logic operations and make decisions in a way not unlike electronic devices do. A phenomenal step in this direction has been the recent development of CELLO (http://cellocad.org/), a platform to design genetic circuits that perform given computational operations, which the user can connect to sensors (the inputs) and cellular functions (the outputs). The user simply provides the DNA sequences for the input promoters (the sensors), data for their ON/OFF signal strengths (in standardised units) and then connects the output promoter to the desired cellular function. More developments in this area are expected to happen in the not so distant future.

Storing and managing information

While, as argued above, the 3 core fronts of the biological standardisation challenge involve functional composition, metrology and language, the story would not be complete without addressing the issue of data management. The existing Repository of Standard Biological Parts (http://parts.igem.org) has already a good number of its listed items associated to datasheets that are similar to those widely used in engineering. A more professional platform in the same realm is the one developed at the Imperial College London under the denomination DICOM-SB (http://synbis.bg.ic.ac.uk/dicomsb/), which is inspired by the highly successful Digital Imaging and Communications in Medicine (DICOM) standard. This system captures all the data, metadata,

and protocol information associated with biopart characterisation experiments. The platform can accumulate and process large amounts of data and includes services orientated towards interoperability and automatic exchange of information between different modalities and repositories, for example, it has been designed to be compatible with and complementary to other standards in SB, including SBOL (see above). An overarching initiative for managing and safeguarding the increasing volume of data being generated by publicly funded research is ELIXIR (http://www.elixir-europe.org), a European-based platform that includes Systems Biology and Microbial Biotechnology community groups. The infrastructure coordinates, integrates and sustains bioinformatics resources across its member states and enables users in academia and industry to access vital services for their research. A considerable focus is placed on interoperability: ELIXIR encourages the life science community to adopt standardised file formats, metadata, vocabularies and identifiers. This helps both humans and computer software to discover, integrate and analyse (big) data, and this objective is brought about by an Interoperability Platform: a group of experts drawn from across Europe, although with a global perspective.

Enabling standards for increased biosafety and easier risk assessment

Since the early days of genetic engineering, biosafety concerns have been brought up and discussed. While we acknowledge that the present risk assessment methodologies are appropriate for assessing potential risks of contemporary SB activities and products, we agree with a recent opinion by the European Commission's Scientific Committee for Emerging and Newly Identified Health Risks (SCENIHR) about research recommendations for risk assessment in SB. The SCENIHR suggested several improvements be made to ensure continued safety protection proportionate to risk, while at the same time enabling scientific, technological and socio-economic advances in the Knowledge-Based Bio-Economy (KBBE). The SCENIHR opinion lists 5 major starting points

for improvement: [i] support the characterisation of the function of biological parts and the development of computational tools to predict emergent properties of SB organisms; [ii] streamline and standardise the methods for submitting genetic modification data and genetic parts information to risk assessors; [iii] encourage the use of GMOs with a proven safety record as acceptable comparators for risk assessment; [iv] aim to ensure that risk assessment methods advance in parallel with SB advances; and [v] support the sharing of relevant information about specific parts, devices and systems with risk assessors. The recommendations of SCENIHR were made to cover a period defined as the next 10 years (beyond which any scenario might rather qualify as science fiction in this field). For this period SCENIHR was concerned that a lack in the support of standardisation on how to obtain and share risk assessment data could lead to an upcoming bottleneck for real world applications of SB. SCENIHR also recommended the support of research and development of novel types of biocontainment strategies (sometimes called i.e. genetic firewall, intrinsic or semantic biocontainment) to add an additional level of containment and safety for real world applications, such as medical use in humans, industrial biotech or large scale agri- or aquacultural deployment. These types of containment strategies have the potential to increase the control over horizontal gene flow and environmental persistence by altering fundamental characteristics of living systems, such as the biochemical compounds of key biomolecules or even the genetic code.

Conclusion

Owing to Systems and Synthetic Biology, modern Biotechnology is becoming more and more comparable to authentic (not just metaphoric) engineering. We advocate standards as the brokers of a new type of Biotechnology which moves quickly and responsibly from laboratory experiments to large-scale processes Simultaneously, we argue that early involvement of the public, amateur biologists and other stakeholders will help steer the direction of technology in socially acceptable and responsible ways.

Further reading

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C3_ WHAT IS NEXT?

Identification of challenges ahead

Standardisation in SB is a hot topic. Several ongoing initiatives such as BioRoboost (http://standardsinsynbio.eu/), the Joint Institute for Metrology in Biology (jimb.stanford.edu/sbsc/) and SynbioLEAP (synbioleap.org), have emerged to address the same issue: the insufficient implementation of standards. Standardisation can be driven by public acceptance/market forces (*de facto* standards), directly ordained by law (*de jure* standards) or, most commonly, arise from the combination of legal/technical requirements and recognition by potential operators since, in general, the broader the applicability of a format, the greater its market.

In this context, the conceptual framework of SB aims to make biology easier to engineer by applying principles such as modularity, orthogonality, chain production and reproducibility. Moreover, the rapid advances in wet and computational tools for genome editing, metabolic design and *in silico* modelling are opening new opportunities for genetic programming that could not have been anticipated even just a few years ago, and are allowing engineers to tackle increasingly complex engineering objectives. The growing demand for scaling up such technologies raises the issue of what is needed to make them work at an industrial scale. We identify the following challenges:

1. Becoming a truly engineering discipline. Following the path of other branches of engineering, the establishment of standards appears among the key objectives of contemporary SB—and eventually of the life sciences as a whole—as a prerequisite for applications such as bioremediation, biomedicine, bioenergy, novel chemicals, innovative materials and cellular factories.
2. Interoperability vs. flexibility. One bottleneck is the widespread and incorrect assumption among many researchers in the life sciences that standards may increase interoperability but necessarily limit flexibility—which is obviously important for any creative research. Rather, good standards will increase people's flexibility and creativity because it will make it easier for them to achieve their scientific objectives. A separate challenge is identifying specific systems and operations that need to be standardised, and then navigating the minefield of personal interests that typically inhibit agreement on a given format or language. As Murray Gell-Mann quipped, "a scientist would rather use someone else's toothbrush than someone else's nomenclature". Scientists and engineers will adopt standards only when they add value to their efforts to overcome the often steep costs of adoption.
3. Digital vs. flexible. One way to alleviate this problem is by redesigning regulatory components to behave more digitally, but ultimately, we may need to revisit information processing in/by biological systems with other formalisms, either existing or yet to be developed, that go beyond Boolean logic.
4. The human factor: the need to reach consensus. Standards are tools, not goals by themselves. The most conspicuous technical challenges include standardising simple biological parts, devices and circuits, chassis, metrology, descriptive languages (including graphical representations) and software tools. But the complexity of the endeavour also asks for the creation of a network of SB practitioners that share and evolve these standards together.

5. Where are we (and where do we want to be)?

   Not every research or technological activity is necessarily carried out with standards but, in the case of SB, there are already some examples of standardised activities/measurements that have been identified and proposed as paradigmatic: *in vitro* and *in vivo* experimental set-ups, SB toolbox generation and computational research (Tas et al., 2020). These include the PoPS, biological parts and cloning tools, as well as genetic, protein, metabolic, systems-scale and data-sharing computational standards.

The adoption of a standard is known to follow the so-called adoption curve, in which the process of adoption over time follows a classical normal distribution or "bell curve". We have identified that SB standards are largely still in the innovator phase of the curve, but some cases have clearly progressed to the early adopters or even early majority segments. Figure 2, adapted from Beal et al. (2020) displays the "maturity" of different standards plotted on an adoption curve: SBOL, SEVA, MoClo, and modular cloning (Figure 2).

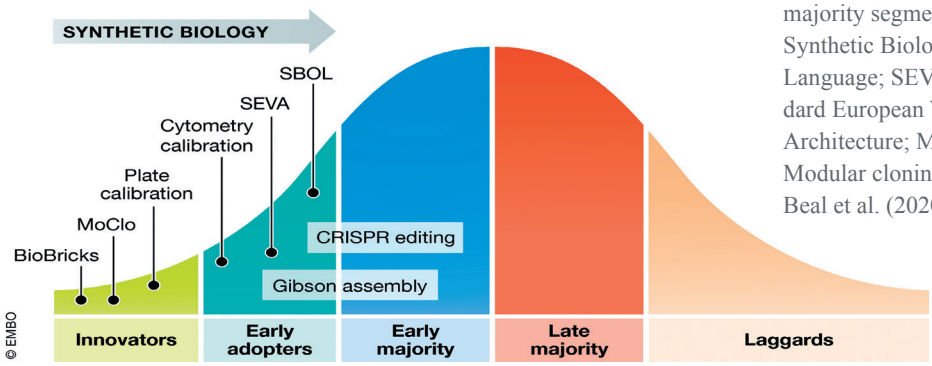

Figure 2. Adoption curve of/for biological standards. Illustrative examples of the position of SB standards along the technology adoption curve: SB standards are largely still in the innovator phase but with a few examples having progressed to the early adopters or early majority segments. SBOL, Synthetic Biology Open Language; SEVA, Standard European Vector Architecture; MoClo, Modular cloning. Source: Beal et al. (2020).

An interesting point is the following question: what are the limits we should impose on standardisation? Tas et al. (2020) address this question and simply suggest renewing standards when necessary (i,.e. Biobricks) and focusing on the integration of the standards with other standards. Since both improvability and

connectability are applied to all standards, it seems reasonable to conclude that standardisation should be applied to SB as far as possible, provided that the benefits of standardisation overcome the hurdles of non-standardisation over the long term.

Conclusion

We argue that the promise of SB for the benefit of global society and industry will only be met if significant advances are achieved on the standardisation front. To this end, it is not only essential to overcome national/political barriers and particular interests of given research groups, but also to gather key players in a permanent forum with the aim of making biological standards one of the ingredients of the 4th Industrial Revolution. Standards in biology will be used provided that they have intrinsic properties such as robustness, ease of use and context independence. But the key to success is the merger of technical consistency and scientific soundness with legal requirements and consensus among end users. This goes beyond the realm of research and tackles sociological and cultural issues that have been traditionally alien to the conversation. If this can be achieved, the benefits for SB and for society at large will be impressive.

Further reading

https://www.embopress.org/doi/full/10.15252/embr.202050521

Tas H, Amara A, Cueva ME, Bongaerts N, Calvo-Villamañán A, Hamadache S, and Vavitsas K (2020). Are synthetic biology standards applicable in everyday research practice? Microb Biotechnol 13(5), 1304-1308. doi: 10.1111/1751-7915.13612.

PART 2
TECHNICAL CORE

C4_
METROLOGY

Overview

Metrology is the science of measurement. Standardised measurements are a foundation for the physical sciences and engineering; they enable uniformity, comparison and quantitation. The international system of units (SI; System Internationale) has seven base units from which all other values can be defined. They are now all defined by physically measurable phenomenon, but this was not always the case. For many years two of the most important units were defined in relative terms: the metre was defined by reference to a physical metre made of lead and held in Paris, while time was relative to the planetary movement of Earth. While the relative definitions are good enough for everyday use, a great effort has been made in these definitions because their precise values are critically important in the physical sciences (https://www.bipm.org/en/measurement-units/).

In biology, some of these base units apply, such as time, temperature, length and moles (amount of substance) and where these intersect with biological measurements they are of course applied. So biological structures are measured in terms of appropriate length units and chemical reactions defined in terms of concentration (moles/volume), time and temperature. For many years this approach has in fact served biochemical study rather well when functional study was based on reductionist studies of pu-

rified biological components. But, in more recent times, biology has moved towards more holistic systems approaches. As cellular based measurements have become more sophisticated, using microscopy, fluorescence and mass spectrometry, so it has become more difficult to ground these measurements in SI based units.

Many of these measurement techniques report in relative units, but to make matters even worse, they are relative only to the machine on which the measurements were made and the settings and operational characteristics of that particular day. So whereas relative units like the original metre are standardised back to a specific reference standard, the output of the photomultipliers that provide the signal in fluorimeters are not calibrated in candelas (the unit of luminosity) and furthermore the output is dependent on other settings, like wavelengths (frequently determined by filters that are also not well defined in performance terms). So fluorescence measured by a plate reader or microscope is relative only to itself.

Consider then, the ambition of SB, which is to apply engineering principles to biology. The group of Chris Voigt at MIT has done an outstanding job of characterising many biological parts like promoters (which control gene expression) and from these characterised parts creating functional logic circuits inside cells that are analogous to electrical logic circuits (Nielsen et al., 2016). They have done all of this using fluorescence measurements from plate readers. While all of their measurements are internally consistent, enabling them to develop this platform, the lack of standardisation in the measurement means that it is difficult for other labs to either apply their measurements in their own lab or even compare against their own measurements. Imagine the electronics industry trying to work without Volts or Amps, and that is about where SB is at.

Towards metrology standards for biology

In biology, absolute units could be envisioned that describe the functional processes at the heart of the CD. For instance transcription (gene to messenger RNA) could be described by PoPs,

while translation (messenger RNA to protein) could be described by RiPs. While it would be desirable to understand and measure these, after 10 years of effort in SB it is painfully apparent that such measures remain a highly specialised research endeavour and are not practical for the everyday measurement of SB parts, devices and systems.

Relative units are often considered to be an inferior form of unit since they are not defined in absolute terms. However, they are widespread and, like the metre, have provided an essential basis for metrology and its development. While we have been unable to define a useful metrology basis for PoPs and RiPs, thanks to the discovery and development of fluorescent proteins with a wide range of colours, we have a pseudo-measure for the processes that we wish to follow in cells. Although far from perfect, and subject to a number of significant limitations, they nonetheless present a powerful, useful and easy method for following relatively complex biological behaviours including sophisticated gene circuits and dynamic responses. Despite their widespread adoption and use, they have for the most part remained as dimensionless terms. This creates significant problems in understanding the significance and comparability of data.

A key method for creating a relative unit is to develop a standard reference material that can be used to calibrate measurements back to the reference (i.e. a ruler is a calibrated stick that we used for comparison of the item under study). Calibration of fluorescence requires standard curves of reference materials, against which experimental measurements can be compared, and hence converted into calibrated units. This enables direct comparison of data generated by different instruments and different laboratories. The principle of using reference materials has been demonstrated by the International Genetically Engineered Machine (iGEM) interlab studies (Beal et al., 2019; Beal et al., 2018), which demonstrated that both plate reader and flow cytometry data can be calibrated and report the same value of fluorescence per cell to within an acceptable margin of error.

The flow cytometry calibration is based on fluorescent beads that are supplied as National Institute of Standards and Technology (NIST) certified reference material and which cover a wide range of wavelengths (Beal et al., 2019). This approach is also very versatile in that it can be applied to both mammalian and microbial cells and since the measurement is by definition per cell, no correction for population is required.

Plate readers are widely used for the measurement of fluorescence and absorbance as they can measure across 96-well plates, making measurement efficient across numerous samples, and crucially they enable continuous measurement for time-based studies. However, there are no such certified reference materials available for plate readers. The iGEM study demonstrated that a solution of sodium fluorescein provided an excellent reference material for calibration for Green Fluorescent Protein (GFP). A limitation of this study was that the reference was not certified nor commercially available. Repetition by an independent laboratory would therefore require the reference material to be remade, with associated errors. It would also be beneficial to extend this approach to fluorescent proteins of other wavelengths in the blue and red range. In principle it should also be extended to cover any fluorescent protein with compensation approaches, such as those used in flow cytometry, to enable multicolour quantitation. A further challenge in this area is to extend calibration to microscopy.

There is thus an opportunity to extend plate reader calibration to other fluorescent proteins and a challenge in developing routes to enhance the uptake and acceptance of the standards, as well as in making suitable reference materials available to facilitate this. Reference materials should include reference dyes as well as genetic constructs and cell types for validation. Further interlab studies to enhance capabilities in this area are a priority.

Cell number is another critical measure in understanding cellular behaviour and is particularly pertinent to the measurement of microbial cell cultures in SB. Once again, plate readers are

widely used to follow cell growth at the population level, rather than individual cells. Typically absorbance at 600 nm (Abs_{600}) is used as a proxy for cell density, since the turbidity of microbial growth solutions scatter this wavelength of light. Absorbance is usually based on the physical absorbance of photons by a species and this gives a linear correlation to concentration based on the Beer-Lambert law. However, since cell density is based on scattering, the fact that this light is not actually absorbed means that the Beer-Lambert law does not apply and the phenomenon is based on the physical geometry of the instrument.

Silica beads scatter light in a similar way to microbial cells (Stevenson et al., 2016) and it has been demonstrated that they can act as a as a proxy for cells in Abs_{600} measurements. Silica microspheres thus provide a useful calibration reference material and it has been demonstrated by the iGEM interlab study that they provide a consistent and useful proxy for cell number. Fluorescence plate reader data normalised against fluorescein and silica beads enabled data in calibrated units of MEFL/Particle that correlated with the MEFL/Cell derived from flow cytometry (Beal et al., 2019). The demonstration of comparable data across different measurement devices and groups was a powerful demonstration of the applicability of normalised units.

There is currently a knowledge gap relating to the universality of this approach, its applicability to other microbial strains and sources of variance associated with changes in cell size during growth. However, there is both a need and an opportunity to develop certified reference materials for Abs_{600} calibration and to understand the relationship to cell size and growth phase.

The quantitation of RNA would help deconvolute the transcription and translation processes, whereas fluorescent proteins can only provide a relative measure of the combination of these two processes. RNA sequencing (RNASeq), has the potential to provide calibrated measurements of cellular RNA (Gorochowski et al., 2019) and it is preferable to reverse-transcriptase PCR (RT-PCR) since it provides whole cell information, and although it

can be highly quantitative and high throughput, it cannot easily be used for dynamic time-based studies. Since it is a whole cell approach, RNASeq can also provide a global view of cellular regulation when constructing complex SB systems or biosynthetic pathways. It is thus a critical workflow that will become increasingly important, especially with the availability of low cost sequencing platforms like the Illumina iSEQ 100 and Oxford Nanopore MinION.

The development of DNA microarrays created the first technology for the quantitation of whole cell RNA. Early on in this effort the industry manufacturers realised that that their results were not reproducible and could not be reconciled across different sites. This led to the External RNA Controls Consortium (Baker et al., 2005) that developed a spike in reference material to enable RNA quantitation which has led to their adoption and development of methods for validation (Pine et al., 2016). Their application to RNASeq has demonstrated the ability to facilitate comparable analysis across samples, protocols and platforms (Jiang et al., 2011).

This is a key area for development and one in which we can learn from these previous advances and reference materials. Pipelines for the routine analysis of bacterial RNASeq data will be made available on a Galaxy platform. An interlab study has been initiated to assess and further develop the applicability of standard pipelines for bacterial RNASeq data analysis, so this is a dynamic space that is rapidly evolving.

Conclusions and recommendations

Recommendations for practitioners

The key message for practitioners is to care more about quantitation of your data. Imagine complete comparability of your data across time and equipment. Not only that, but if this was widely adopted, you could compare, in absolute terms, your data with data produced in other labs. The protocols for adopting these methods are available, as well as software tools to

assist with the calibration process, like FlopR for plate reader data and TASBE for flow cytometry. The sooner the effort is made to adopt calibration standards the sooner SB will be able to start becoming a true engineering discipline.

Data reproducibility and comparability is critical for science and the biological sciences have been lagging behind, with large amounts of published data not being reproducible. There is a pressing need for improved reporting of methods, with the methods sections of publications generally not being very fit for purpose. The adoption and reporting of Standard Operating Procedures (SOPs) would, in combination with improved metrology standards, be transformative. Innovative new approaches are emerging for this type of activity, such as protocols.io and the Journal of Visual Experiments. It is incumbent on us all to improve the reproducibility of the science that we practice.

Recommendations for policymakers

When considering standards for SB, it is important to understand the pathway for standards development and what might be considered different 'levels' of standardisation. At the lowest level, we can consider protocols and best practice guidelines while at the highest level there are community-agreed standards and certified ISO standards. It is important to recognise that, for the majority of academic researchers, best practice is sufficient. Such best practice and protocol development in the form of SOPs will also enable more direct translation into industrial settings. ISO standards are uniquely important for industry where performance of critical workflows is dependent on them. The lifetime for development of ISO standards runs well beyond the lifetime of BioRoboost, but there is an immediate and important opportunity to focus on best practices and protocols as the foundation for standards development.

Standardisation of metrology used in SB was highlighted very early on by the field as being of critical importance. It is therefore disappointing that this field has not progressed much since

that time. There are a number of factors why this has been difficult to resolve, one of which is the technical complexity, but as highlighted by other sections of this white book, issues of standardisation go beyond the technical. The reward system in academic research has not been well setup for success in this field: it is a low priority both for high impact journals as well as funding panels and reviewers (though not necessarily the funding agencies themselves). This has made it difficult for the few researchers committed to improving standardised metrology to make headway. Even where progress has been made, adoption by the community has been low and a requirement for measurement standards by journals will probably be a key factor in widespread adoption. But for this to happen, the reference materials and tools need to be better developed.

C5_
CHASSIS [2]

What is a SB chassis?

The concept of the chassis as a defined, reusable, biological frame where non-native components can be plugged in and out to create new functionalities, lies at the boundary between frontline bioengineering and more traditional recombinant DNA technology. The term evokes the basic frame of a car to which a number of components can be added in response to specifications and/or customers' desires. The word chassis (and the powerful metaphor embodied in it) has been incorporated into the habitual discourse of SB as an engineering discipline. The prevailing meaning of chassis is that of a more or less improved host for genetic constructs whether in bacteria yeast, fungi, archaea, animal or plant cells. But the meaning of the word has thus quickly undergone a process of polysemic diversification to the point that the metaphor is kept in all cases but the precise meaning has become increasingly blurred, thereby the need of clarification and even a definition of the term that end-users can understand without any ambiguity. A proper definition of chassis can ease regulatory roadmaps to industrial and regulatory acceptance, as SB agents start falling under the radar of agencies that that provide risk assessment advice on products used for the agri/food/feed chain

2 This section is an abridged and updated version of de Lorenzo *et al.* (2021).

(i.e. the European Food Safety Authority or EFSA) and that even have regulatory authority (i.e. the US Food and Drug Administration or FDA).

The discussion about chassis would certainly benefit from having objective criteria for distinguishing specialised carriers of synthetic constructs from mere recipients of cloned DNA. As a first approach, one can describe a SB chassis as *an engineerable and reusable biological platform with a genome encoding a number of basic functions for stable self-maintenance, growth and optimal operation but with the tasks and signal processing components growingly edited for strengthening performance under pre-specified environmental conditions.* It is important to note that the key of the definition is optimal *performance*, not *minimised genome size* (although deletion of unnecessary functions will certainly cause a degree of genome reduction).

The quest for the optimal chassis has been addressed from various perspectives. In one case, the idea is to start with a well-characterised bacterium (e.g. *E. coli*) and then delete the parts of the genome that are not necessary for growth in a given environmental context. For the time being, some of these minimised *E. coli* strains are the best available chassis for the implantation of new genetic circuits. Note that the definition above implies optimal chassis per specific target environment and tasks therein. The concept thus entails that there may not be a best possible version of these microorganisms, but instead one would find a growing series of ed upgraded variants.

Difference between traditional carriers of recombinant DNA and SB agents

The definition above embodies the idea that such chassis stem from well understood and characterised natural organisms, which have been genetically streamlined [i] to build, maintain and amplify components necessary for deployment of SB systems and applications but also [ii] to ease genetic and metabolic interventions and reduce their adverse effects. To this end, such

a chassis should be endowed with natural and knocked-in features suited for facilitating optimal performance in specific settings. For this to happen, they should be amenable to an ample and practical engineering toolbox that allows construction and deployment of genetic devices/circuits with a minimum of engineering steps and thus avoids surprise interactions with host functions.

Obviously, these criteria overlap with properties already present in many types of bacteria that can host recombinant DNA and be genetically programmed for a variety of fundamental or biotechnological purposes. However, we argue that a SB chassis is more than that: to go beyond being a simple recipient of rDNA and move towards a *bona fide* SB agent chassis status, engineered microorganisms should have progressed through a well-defined roadmap in which each milestone has unequivocally defined properties. Such a "chassiness" roadmap will help scientists to demarcate more rigorously what a SB chassis is but, more consequentially, it will also help regulators and policymakers. This is because a (limited) number of standardised microbial platforms —along a well-defined and measurable *chassiness* itinerary— will enable a more transparent and robust examination of regulatory fulfilment while simultaneously lifting regulatory burden via streamlined decision-making when it comes to industrial applications or environmental release.

The roadmap from being a rDNA host to a fully-fledged certified SB chassis

The itinerary proposed in terms of information and modifications needed for upgrading a promising environmental isolate to a bona fide standardised SB chassis is shown in Figure 3. Any (preferably non-pathogenic) environmental isolate able to capture exogenous DNA, through transformation or conjugation, and stably maintain it and for which a minimum of genetic tools is available, can be tagged in principle as a recombinant DNA (rDNA) host. The historical example of this category

is *E. coli,* but now there are dozens of species amenable to a suite of genetic manipulations, including pathogens that are handled under controlled laboratory conditions. But to become a true chassis, the biological host should be agreeable to and optimised for accommodating complex genetic devices and deploying their encoded properties under specified operational conditions. For this, additional requirements are needed: the complete genetic complement should be known and advanced genetic tools for deep editing be at hand. This should result in a profound knowledge of the energy metabolism (typically through reliable metabolic models), stress resistance and sensitivity to antibiotics and phages.

Knowing the ratio of synthetic/engineered DNA vs. natural genetic complement is straightforward in these cases. Furthermore, genetic and evolutionary stability of the resulting constructs is a most desirable trait. This could be enhanced by engineering circuits that somehow punish mutations in the genetic implants or by making cells deficient in endogenous recombination systems. This, in turn, requires specific genome editing methods that do not rely on recombination, such as targetrons or base editors. Up to that point, one can consider a large number of species and strains that can qualify as, or become, SB chassis (Table 2). Things get more restrictive, however, when strains are destined for actual, large-scale biotechnological applications, as they must meet additional specifications that are not that important in the laboratory or in academic settings. Most of them deal with safety and efficacy issues, which need to be addressed to overcome Environmental Risk Assessment (ERA) criteria and gain a green light by regulatory agencies. Properties of interest to this end include antigenicity and Horizontal Gene Transfer (HGT) —either as donors or recipients of DNA. For some specific applications, containment of the strains themselves or at least barriers to HGT to/from them are necessary, while in others propagation of beneficial traits to the surrounding natural community might be desirable, depending on the goal.

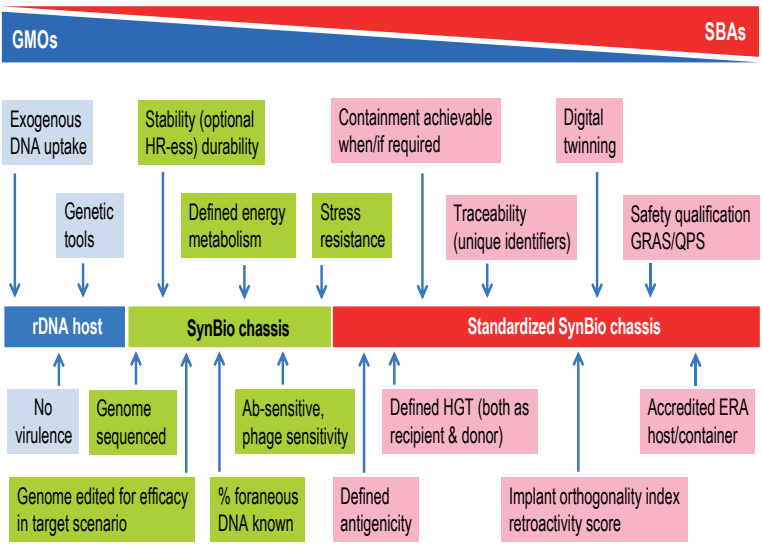

Figure 3. The roadmap from environmental isolates to fully-fledged standardised SB chassis and from GMO (genetically modified organism) to SBA (SB agent). The scheme indicates the nature of the information that should be available for each category of strains. Note that there is not a defined boundary between GMOs and SBAs. One important aspect of standardised chassis is their digital twinning that can be implemented through DNA barcoding as explained in the text. The final product of the process should be an effective and ERA-acceptable host of SB devices—or in general rDNA constructs. Source: de Lorenzo et al. (2021).

The scheme shown in Figure 3 also embodies the difficulty in distinguishing SB chassis as something completely different from GMOs, as there is a clear continuum between the two. At the same time, it is not realistic to have an infinite number of chassis. Instead, it would be more practical to define a limited number of them for specific uses or environments that are thoroughly, standardised, characterised and given a certain safety score.

Barcoding as an avenue to ease traceability and manage contingencies

There are many proposals for genetic firewalls to contain genetically engineered organisms and SB agents. Current methods, however, do not allow detection of escape events occurring at frequencies below 10^{-12}, which is not enough to prove Certainty of Containment (CoC) for an environmental release. The scientific question about CoC is a very interesting one, but alas, achieving it is not yet in sight. We argue that barcoding can meet a considerable number of safety issues. Once decoded, barcodes can deliver the best available information for specific constructs such as their origins, parentage, safety, and modifications im-

plemented in them, and serve as a complementary approach to any kind of containment measures. Although this is indicated for the use of publicly retrievable barcodes, the abovementioned argument also applies to hidden barcodes (i.e. watermarks or steganographic data). Barcodes will not only make traceability simple, but they will also assign a non-ambiguous cipher to the increasingly improved versions of the same chassis. This is the case with the Operating Systems (OSs) of computers and phones as well as version control for updated variants of the same software. It could be possible to have a series of standardised chassis derived from the same original strain but barcoded to design version 1.0, 1.1, 2.0, etc. In this respect, there is much to learn from the way the computer industry has dealt in the past with similar challenges. In both cases, standardisation and version control increase safety, enable tracing versions and sorting Intellectual Property (IP) issues and should thus ease regulatory frames. Ultimately, SB would benefit from adopting digital twinning technologies which have had an enormous and positive impact on other industries.

Conclusions and recommendations

The boundaries between traditional GMOs and SB agents are quite blurry and objective criteria to distinguish them is difficult. We propose some possible avenues to tackle the issue, but regulations ultimately boil down to numbers and thresholds that are arbitrarily set by the corresponding authorities. One possible approach could involve quantification of the % of genomic DNA that has been inserted/deleted in the SB agent in respect to the ancestral host. Once such a level is agreed, the strain at stake would be a GMO if the % goes below the figure and an SBA above the mark. But other criteria are equally possible or desirable: % or number of biological parts implanted, number of manipulative steps that were necessary to engineer the agent of even the share of new information implanted in the microorganism. In any case, the incorporation of SBAs to the biotechnol-

ogy industry of the future will demand a dramatic change in the way we run environmental risk assessment from an individual basis to focus on a limited number of well-accredited chassis along the lines herein presented.

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Table 2. A sample of proposed microbial chassis for SB.

Genus / species	Qualities of interest
Mycoplasma sp.	Small genome, vehicle for delivering therapeutic activities to the lung
Escherichia coli	Laboratory work horse, recombinant DNA host, abundant genetic tools
Pseudomonas putida	Tolerance to environmental insults (solvents, redox stress), platform for metabolic engineering
Bacillus subtilis	Laboratory workhorse, easy recombineering, efficient secretion systems
Corynebacterium sp.	Long time applications in industrial biotechnology, large-scale production of amino acids
Saccharomyces cerevisiae	Laboratory workhorse, easy genetic manipulations, optimal eukaryotic metabolic engineering platform
Pichia pastoris	Large-scale production of recombinant proteins & chemicals
Synechocystis/ Synechococcus	Photosynthetic organisms, CO_2 fixation, emerging metabolic engineering
Streptomyces sp.	Diverse secondary metabolism, production of antibiotics, efficient secretion systems
Vibrio natriegens	Super-rapid growth, easy to engineer, host of recombinant DNA constructs.
Lactobacillus sp	Platform for engineering in situ production of bioactives by designed probiotics
Alteromonas sp	Delivery of biodegradative and bioremediation activities to marine systems
Rhizobium sp.	Agents for targeting plan roots and designing new symbiotic systems
Yarrowia lipolytica	Biotransformations with apolar substrates and products
Halomonas sp	Growth at high densities in non-sterile seawater. Easy genetic manipulation

C6_
YEAST

As our understanding of genetic bio-circuits has improved, there has been push towards standardising the bio-design process. This involves the creation of a library of characterised modular DNA parts which could be joined to form more complex genetic circuits as appropriate for the desired application (Beal et al., 2020). This would enable the bio-designers to focus on higher-level experimental design and further socioeconomic impact rather than being preoccupied with the technical details of building complex circuits.

Not surprisingly, the majority of these standardised DNA libraries have been performed using the prokaryote *E. coli* due to historical reasons, as well as the advantages of having an easy cultivation and detailed genetic manipulation protocols (Pontrelli et al., 2018). Hence, many standardised languages and DNA toolkits have been initially developed for such chassis. Although *E. col*i still dominates the area of SB, the need for more sophisticated genetic designs coupled with certain limited capabilities of *E. coli* led to the necessity of alternative chassis organisms and their respective toolkits such as *Saccharomyces cerevisiae* (Adams 2016). *S. cerevisiae* is one of the most studied eukaryotic model organisms with biochemical and genetic features well characterised leading to a broad-range biotechnological applications (Jouhten et al., 2016). *S. cerevisiae* has been employed for most common top-down bio-design strategies, but remarkably it has also been selected for ambitious bottom-up bio-design initiatives such as the

Sc 2.0 project (Chi et al., 2019) in which the entire yeast genome is replaced with a heavily edited, and totally synthetic version.

The SB community focusing on the engineering of *S. cerevisiae* has been at the forefront of developing standardised DNA libraries and many fully-characterised SB toolkits. The impact of these toolkits has gone beyond *S. cerevisiae*, as they have been successfully modified to cater for other industrially-relevant yeast species such as *Yarrowia lipolytica*, *Komagataella phaffi (Pichia pastoris)* and *Kluyveromyces marxianus* among others. Despite this, SB advances are happening quickly, especially in automation, miniaturisation, genome engineering, emerging yeast chassis and microbial consortia. Therefore, there is still a long journey to go in terms of standardisation to fulfill the full potential of yeast SB.

The scope of this section of the project was to identify best practices through the description of several *S. cerevisiae* DNA toolkits that have had an important standardisation role, highlighting the further impacts of these toolkits. We believe that by sharing the successful impact of these toolkits, best practice lessons could be learnt to be applied to chassis beyond yeast, greatly increasing our effort to standardise SB as a whole.

Community driven Yeast toolkits: iGEM BioBricks and JBEI-ICE

Community-driven DNA libraries for *S. cerevisiae* were among the first efforts to drive the yeast SB community to adopt standards. The key benefit from such approach is democratising the access and building of such libraries, as well as the continuous update and refinement of its bio-parts.

Although iGEM's BioBrick collection is focused mainly on bacteria (i.e. *E. coli*), there is a growing collection of a few hundred characterised *S. cerevisiae* parts available. This makes the *S. cerevisiae* kit (www.parts.igem.org/Yeast) rather small compared to the over 20,000 parts documented in the iGEM Registry. Still, the Yeast BioBricks Assembly (YBA) is one of the early examples of standardisation studies of yeast expression vector assem-

bly (Schneider et al., 2012). Furthermore, the community built up upon this standard to develop better characterised toolkits and more practical assembly methodologies such as the Easyclone Vectors (Stovicek et al., 2015) and EasyClone-MarkerFree system (Jessop-Fabre et al., 2016).

The standardised DNA assembly method is based on the Type II restriction enzymes to create compatible ends with flexible modularity enabling the easy construction of more complex designs. As previously mentioned, the catalog has been built in a participant-based 'get' and 'give' approach which is the key strength of this community driven approach, but this has also led to some quality challenges. While the sequences are confirmed by iGEM, the quality of several of the bio-parts have been found to be questionable (i.e. not functional) in addition to not having any or minimal associated characterisation data.

Similar to the iGEM repository, the Joint BioEnergy Institute Inventory of Composable Elements (JBEI-ICEs) is a repository containing information about biological parts, plasmids and strains (Ham et al. 2012). This community-driven platform (https://public-registry.jbei.org/) currently hosts more than 300 yeast-related plasmids submitted by the community. Although relatively fewer parts are available in this repository, it is quite well-organised as detailed information including graphical applications for part annotations and creator's contact details are found for every single part.

S. cerevisiae DNA Toolkits created by single studies or research groups

In contrast to the community driven approach previously mentioned, several DNA toolkits have been specifically built for *S. cerevisiae* by single laboratories/institutions such as MoClo (Lee et al., 2015), YeastFab (Guo et al., 2015) and GoldenBraid (Pérez-González et al., 2017) among others. One of the key advantages of these studies is that the DNA parts and their effect on expression have been well characterised using a single standardised

methodology, greatly improving the quality of each bio-part and allowing the users to easily compare their performance with confidence. The availability of quality information on the DNA part itself, in addition to how the parts interact in a construct, clearly facilitates the building of more complex bio-circuits.

Among several toolkits developed in this category, the modular cloning system named Yeast Toolkit MoClo (Lee et al. 2015) has been one of the most widely adopted by the *S. cerevisiae* community. It is based on the Golden Gate Assembly method and uses type IIS restriction enzymes to create unique 4-base overhangs for multi-part assembly reactions, further improving the assembly method by allowing up to six parts to be efficiently joined simultaneously in a rapid way. Another advantage is the use of assembly connectors to make it compatible with parts from other toolkits. The toolkit consists of 96 standardised parts, such as promoters, terminators, peptide tags, origins of replication, as well as genome-editing tools all available in a single 96-well plate format (https://www.addgene.org/kits/moclo-ytk/).

As previously mentioned, the toolkit has been further adapted to be used in other yeast species such as the methylotrophic *Komagataella phaffii* (Prielhofer et al., 2017), *Yarrowia lipolytica* (Egermeier et al., 2019) and *Kluyveromyces marxianus* (Morrissey et al., 2015), among other yeast species.

A key limitation of this toolkit is that there are only minimal parts published at this stage, and the nature of the toolkit hasn't allowed for further updates, limiting its flexibility. In an attempt to overcome the limited number of parts available, Guo et al. (2015) developed a standardised DNA construction method called YeastFab where hundreds of DNA parts were characterised and standardised, for the easy and rapid assembly of multi-gene pathways (www.addgene.org/search/catalog/plasmids/?q=YeastFab). In order to overcome the lack of continuous updates from Yeast MoClo, a Yeast GoldenBraid cloning system was developed containing four integrative plasmids, nine promoters, eight mitochondrial targeting signals, one N-terminal tag, three terminators and two dominant selective markers (Pérez-González et al., 2017). The GoldenBraid

collection of standardised parts and tools are available and continuously updated online (https://gbcloning.upv.es) where detailed experimental tutorials are also provided and updated.

Conclusions and recommendations

The studies aiming to develop toolkits and DNA assembly methods for various yeast species have greatly contributed to the standardisation of the related DNA parts and methods. This has allowed *S. cerevisiae* to become an important SB chassis, in addition, to allow emerging new yeast species to benefit from the previous standardisation knowledge acquired.

The BioBrick yeast library, while extensive and full of potential due to the community-driven nature of its development, still lacks standardisation in terms of parts characterisation and quality highlighting the more informal nature of this repository. On the other hand, the level and quality of information available per part are higher and more explicit in newer toolkits created based on single studies or laboratories such as MoClo YTK, YeastFab, or GoldenBraid, which have been widely adopted by the community due to their extensive and trustworthy characterisation data, easy assembly, and practical access through Addgene and similar repositories.

Keeping the toolkits and parts updated is a necessity for standardisation. Even though some toolkits like MoClo have been relatively widely used and have been adapted to multiple species, they lack version updates. At this point, BioBricks kept in the iGEM catalogue stand out as the parts are updated regularly. This necessity has been catered by newer toolkits such as the GoldenBraid, which now includes version updates providing the latest information about part collections and methods.

The relative maturity of the yeast community in terms of the numbers of available SB toolkits and the adoption of standards by the users are greatly contributing to the translation of yeast SB into real products and applications in the next decade, and their lessons learned will be of great utility for other SB communities and emerging chassis.

Recommendations for practitioners

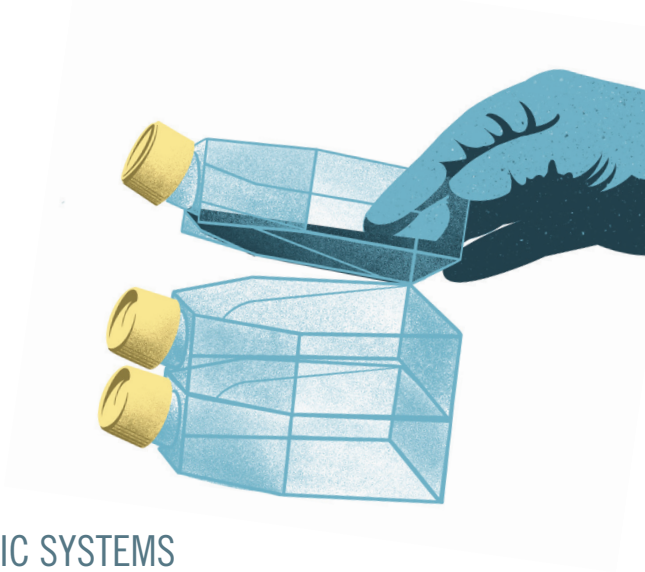
- Increase the effort to validate/curate community driven repositories.
- Provide continuous updates to SB toolkits from single studies or laboratories.
- Develop adapters or "connectors" to enable different toolkits to work together.
- Access to the toolkits should be promoted at a lower cost from single repositories, making options available to lower income countries to get access to such toolkits in order to establish such standards.
- Journals should promote and reward the use of standards.

Recommendations for society

SB is an engineering discipline that uses standardised tools to bring efficiency to the construction of novel and safe bio-systems which could contribute greatly to humankind's progress towards sustainable, carbon-responsible manufacture of a myriad of products. It is recommended to promote, since early stages, science communication activities about the benefits of SB and the need to develop novel standardised tools, so that the public can make informed choices about how SB becomes deployed across society.

Recommendations for policymakers:

- Funding should be available to develop newer DNA toolkits, while maintaining and updating the DNA repositories (especially if the toolkit comes from a single study or research), verifying the quality of the DNA parts of a toolkit (especially if it is a community driven library) and sharing the DNA toolkits, especially for lower income countries.
- Use of standards to engineering biology should be included in the curriculum of SB, Bioengineering and Bioprocess Engineering courses among others.



C7_
OTHER EUKARYOTIC SYSTEMS

The application of SB to mammalian systems lags significantly behind than microbial systems. This reflects the significantly greater technical challenges of implementation within the more complex context of the mammalian cell, and also the longer journey to commercial application that this necessarily engenders. Nonetheless, mammalian synthetic biology (MSB) holds significant promise for high-value applications within modern medicine and cellular agriculture. Medicine is beginning a journey away from reliance on small molecule treatment of a disease class towards biotherapeutics: complex biomolecule/bioentity treatment tailored to an individual's condition. For MSB, the power of the technology in providing new-to-market technologies and opportunities can already be seen. An MSB medical product may either be a biomolecule or a biomolecule complex synthesised within an engineered cell (i.e., AstraZeneca's COVID-19 vaccine produced in a human cell line), or an engineered cell itself (i.e., Novartis's anti-cancer product Kymriah in which the patient's own T cells are engineered to CAR-T cells that recognise and attack the cancer). Similarly, cellular agriculture seeks to take food & feed production away from industrial livestock production, instead growing proteins (animal and fish), fats and tissues in a bioreactor using engineered cells (i.e., the "cultured meat" concept). Cellular agriculture's products primarily target the food/

feed sector, but these products may also impact fine chemical markets as animal-derived chemicals are found in a diverse set of products such as soaps and other personal hygiene products, cosmetics, cleaners, candles, waxes, and lubricants.

An important difference between MSB and much of industrial biotechnology is that the engineered cell itself may be the product, as opposed to a chemical produced by the cell, with the cell in many cases to be administered to or consumed by the public. MSB faces numerous challenges, many of which step beyond the technical. A key challenge lies in supporting technology development so that MSB achieves its full socioeconomic impact, reaching the widest consumer base, at an optimal price, and with the required safety. Regulation and standardisation must be embraced as a powerful partnership to de-risk development, short-cut time to market, and encourage both public and private investment. But their deployment must be sensitive to avoid unintended constraint, procedural delay and potential commercial disadvantage accruing to those subscribing to regulation/standardisation.

For MSB to prosper in Europe, it is essential to institute a diverse, yet coordinated, ecosystem of government regulation and monitoring, paired with standardisation around issues of product safety, quality and provenance, endorsed by non-profit trade associations that, in turn promote standard development and adoption.

Assessing the standardisation landscape for mammalian cell systems

There is widespread recognition of the pragmatism of sharing best-practice, with its power to drive down cost, boost quality and trivialise the mundane. Standardisation has become a central tenet of the "engineering" process. The importance of standardisation to microbial SB is well-understood (as discussed in Chapters 4 & 5); there are numerous areas of need with inadequate technical implementation and/or insufficient (meta)data quality, exacerbating the issue of the genetic or cellular context.

This situation is orders of magnitude more acute for the younger field of MSB as a result of the profoundly more challenging cellular context; their increased genetic complexity is amplified by dynamic epigenetic processes, compounded by their morphological diversity across scales (organelle-cell-tissue-organ) and hamstrung by our paucity of understanding. MSB suffers from a deficit of proposed standards and little consensus on adoption of those that have been developed[1,2]. A significant lack of standardised quantitative measurements and more comprehensive toolsets have been recognised in the MSB literature (Mathur et al., 2017; Black et al., 2017). Addressing these problems holds the prospect of greatly enhancing robustness and impact of MSB research, especially with exponential improvement in computational power and associated bioinformatic tools. With no common, standardised strategy for mammalian protein expression systems available, guidelines have been proposed regarding selection of the approach from the vast number of options available (Hunter et al., 2019). Notable early successes have demonstrated the ability to compose complex mammalian systems with predictable (programmed) behaviour from well-characterised, high-performance parts (Weinberg et al., 2017). Development of MSB standards infrastructure is essential to render trivial the breakthroughs that propel step changes in many important fields, from cellular medicines to protein therapeutics.

There is a particular intersection of need that lies at the nexus of metrology and computational modelling. A well-rehearsed trope for modelling is "rubbish in, rubbish out"; the value of a model's predictions are predicated on the quality of the information input into the model. Bioinformatics and computational modelling across bioscience are hamstrung by the paucity and low-fidelity of available measurement. A notable exception lies in the domain of DNA sequencing that benefitted from co-ordinated investment due to the Human Genome Project and serves as the prototypical outcome. Otherwise, cell metrology is in the very early days of being able to fully address the complexity of cellular measurements, to yield the ability to map the cell's genes

to its behaviour (its genotype to phenotype). In MSB there is a tendency to place stronger emphasis on technological advances aligned with the application of interest, prioritising advances in cellular processes or cell-based therapies. There is an urgent need to establish an infrastructure that supports delivery of quantifiability, reproducibility and traceability in cell metrology (Faruqui et al., 2020). The PubMed assay guidance manual demonstrates that the broader life science community recognises the import of coordination; it has specific sections on reproducibility and standards for biological models (https://www.ncbi.nlm.nih.gov/books/NBK53196/). MSB needs to buy-in to and expand assay guidance, specific to its future requirements. The opportunity exists within MSB to explicitly link standardisation of cell metrology with the contractual and regulatory pressures relating to the product. Not only would this act to protect the consumer from any unintended consequences of an MSB product but it would also mitigate the growing jeopardy that data irreproducibility will compromise promising life science research and undermine the public's confidence therein.

In Europe, the regulation of drugs, and more recently gene and cell therapies, is delivered by the European Medicines Agency (EMA). A well-established set of regulations and enforcement codes generates a partnership between regulator and industry to assess products through their development and into clinical deployment. The medical products arising from MSB will necessarily drop in to this system, but may they also challenge it? There is a future in which our endeavours become increasingly personalised as bespoke cell therapies are engineered to an individual's requirements. This will create tension within a regulatory system that is geared towards regulating products for safe and effective deployment within a population. Such tension may deepen as engineered therapies become multicellular, and possibly hybridised with non-biological elements; would an implantable medical device that integrates engineered cells be regulated by the European Medicines Agency (EMA) or CE-marked by a country-specific Notified Body?

Thus far we have dwelt upon medical application of MSB but an equally important domain is in cellular agriculture. Food security concerns, ethical objections to animal exploitation and the need to reduce the carbon footprint of industrial livestock production are driving interest and investment in the notion of producing animal proteins, fats and tissues in bioreactors using engineered cells. Whilst the food/feed sector is the target of development, it should not be ignored that impact will be felt across many sectors, from the chemical industry (a reduction in availability of meat by-products) to clothing/fashion (biosynthetic leather). Regulation of food products is undertaken by the EFSA, helping to protect consumers, animals and the environment from food-related risks including licensing of GMO introduction to our food chain. Cellular agriculture shifts the goalposts; debate moves beyond how to define GMOs in the light of new gene editing technologies such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and begins to broach deeper philosophical questions around definitions of organisms/species, their genetic provenance and so forth.

Cellular agriculture may be poorly served by the *status quo* with regulations framed with respect to genetically modified crops and animals not adapting easily to this novel unorthodoxy. To illustrate the key role of regulation in this specific segment of MSB, consider the following scenario: a Hungarian cellular agriculture SME leases a Panama-registered bioreactor factory ship, that collects a cargo of amino-acids in South Korea, bioconverts this to beef cell mass using Charolais muscle stem cells that have been engineered with rapid growth transgenes in China, with the bioconversion happening at sea whilst in transit to the "protein" subsidiary of a Dutch food corporation. What genetic and geographic food provenance information should be provided to the consumer? How can the pathogen/toxin-free status of the product be assured? How can technical standards be framed to support contractual safe-guards against reach-through liability across each level of this supply chain?

Conclusions and recommendations

There exists the possibility that the need to take products through regulation (EMA, EFSA and others) can be used to impel the development of standards in MSB. The 21st Century industry which we seek to incubate to broad and deep impact across applications in therapeutics and diagnostics (in humans and domesticated animals) and the ecologically responsible nutrition thereof, mandates coordinated and sustained investment in the development of the necessary portfolio of standards. With the collaborative development of standards between academic and national measurement institutes, the current and projected needs of companies can be met to support their progression to impact in Europe.

MSB presents an exciting set of applications with significant economic potential but also potential societal implications. Industry regulation will clearly be necessary to ensure consumer protection and information at the levels expected within the EU. This will be a complicated landscape to navigate, with management of the interfaces between technologies and the bodies responsible creating potential fault lines. Indeed the merit of a regulatory agency dedicated to SB ought to be considered.

Recommendations for practitioners

Given MSB's immaturity and importance it is important that academia and industry cooperate in this endeavour to:

- Identify mammalian cells as preferred chassis in specific application scenarios. Historical origin and influence of biological sex chromosomes of a cell may need consideration in some circumstances.
- Foster adoption of part/assembly standards through a combination of incentives and imperatives (i.e. journals mandate compliance with specific standards); engage with the Global Biofoundry Alliance to promote mMoClo/EMMA as MSB "assembly standards" in their operations.
- Establish a vehicle to deliver metrological traceability and standardisation. Link the MSB community to the Cell Anal-

ysis Working Group of the Consultative Committee on the Quantity of Substance and develop missing metrology for key tasks in mammalian biotech (i.e., transgene expression, epigenetic modification etc.), determining what to measure, how and with what resolution.

- Empower bioinformatics/computational modelling through a bipartite initiative in data quality grounded in physical, rather than arbitrary, units (Recommendation 1c) partnered with exacting (meta)data standards that mandate capture of a minimal set of essential information.

Recommendations for society

MSB's acceptance must be preceded by careful dialogue to ensure the publics assent. Standards and regulations can act to reassure publics of safety, and also provide clear cues to industry as to that which is permissible;

a. MSB adoption can be de-risked by providing direction to SMEs with bespoke product standards alongside existing Good Clinical Practice (GCP), Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) regulations.
b. Early clarity provided by well-framed standards can act to encourage private investment by creating assurance with respect to the navigation of regulatory processes ("barriers") to access markets.

Recommendations for policymakers

Policymakers' role in compelling responsible development with sufficient alacrity to enable Europe to capture this opportunity cannot be understated. Alignment of existing EU regulatory bodies and national standards organisations with these emerging MSB technologies needs urgent auditing: do they possess the requisite competences? Are they sufficiently agile to respond to, indeed anticipate, need for support in taking new types of genetically engineered product to market? Is there sufficient co-ordination within the EU and to external stakeholders?

PART 3
THE SOCIAL CONNEXION

C8_
STANDARDS AS SOCIAL CONSTRUCTS

Standards and standardisation are often understood abstractly and treated superficially. Standards are explained or described using terms like ‘criteria,’ ‘parameters,’ ‘metrics,’ ‘guidelines’ and ‘rules.’ Units like ‘millilitres’ and ‘microvolts’ remain little more than words and ideas unless examined in relation to tangible things like objects, people, practices and places. The social sciences offer methods for such an understanding.

The social sciences examine standards in their lived realities. They treat standards as coordinated activities carried out by groups of people with objects and materials, in time and place, guided by intentions, with identifiable causes and consequences. A ‘millilitre’ is the product of active individuals (biologists) using physical tools (pipettes) to draw tangible materials (a liquid chemical) to carry out specific action (introducing chemical into a bacterial culture) in an identifiable place (a lab) at a specific time and in the service of a lived undertaking (an experiment) with particular results (the growth of the culture). The result is an understanding of standardisation as a process of social coordination and of standards as the results of collective human practices. In other words, standards are social constructs.

A social scientific approach to standards and standardisation makes them something observable. It reveals them as present and consequential in material ways. It draws focus to how groups

come together, organise their joint actions and maintain their operation. And, it provides insights into the obscure politics of standardisation.

The present document demonstrates and substantiates these claims, while also introducing 'social infrastructures', a concept that helps to identify and study what keeps social constructs like standards in place and how their existence shapes those groups that make and employ them.

People commonly understand and refer to standards abstractly. Consider as an example a millilitre, a standard commonly used in biological laboratories. The term 'millilitre' is itself nothing more than a label. A millilitre can be described as a 'measure' or a 'shared unit.' However, that simply replaces one abstraction with another. A 'measure' is not a material object or practice. A 'shared unit' does not capture the lived practice of sharing. What a millilitre measures—volume—is also an abstract concept, since it does not represent any *particular* volume, but only the general concept.

Abstract understandings lack empirical substance and specificity. They are disconnected from the people who use the standards, the materials used, the practices in which standards are employed, and the spaces and times in which those practices occur. Abstractions offer little insight into the workings of standards. They also provide no guidance for practical engagement, as they are not situated in any kind of place or practice.

Even when given some substance, or considered with regard to material objects and actions, standards are often discussed superficially or portrayed simplistically. A millilitre can be understood as something involved in carrying out a measurement (as one part of an activity). It can be understood as an instruction, such as lines on a test tube (as a kind of visual representation). It can be explained in terms of measured materials (a certain amount of liquid). These descriptions move beyond abstractions, but they still offer little insight into the inner workings of standards and standardisation.

Social constructs

All social constructs share four characteristics. First, social constructs are collective accomplishments. They are products of group activity that cannot be explained by the actions of any single person. Second, social constructs are situated within particular collectives. They always exist within the bounds of a given group. Third, social constructs are contingent on their collectives. They depend on the group for existence and longevity, and reflect the group's distinctive qualities. Fourth, they shape and are shaped by the social collective. Their existence, enabled by the group, also affects the existence and character of the group. Standards display all of these qualities.

As social constructs, standards are products of collective activity: establishing them successfully requires commitment from the community of intended users; putting them to work demands collective engagement in shared practices; and sustaining them requires group fidelity. Units like millilitres were established by groups of people whose collective agreement was actualised by shared practices. And millilitres persist because their user communities sustain their commitment and continue the necessary practices.

As social constructs, standards reflect and shape the collective. For instance, functional specifications, which reflect the group's work, influence the form given to a group's standards. At the same time, an established standard is part of the group and shapes its form. Standardised parts influence the group's material practices and products. Work has to be reshaped to meet standardised procedures and shared parameters, and standardised terminology affects the group's speech, writing and everyday dialogues (Star and Ruhleder, 1996)

Last, as social constructs, standards are situated within the bounds of the collective and entangled in its workings. Once they are fully instituted, standards operate as quotidian parts of everyday activities. As a result, they become hidden within the practices that they support, and what keeps them in place also become opaque.

Social infrastructures

Planning, designing, building, implementing, using, administering and sustaining standards all require social coordination (O'Connell 1999; Mallard 1998). Standardised talk requires using the same terms in the same ways. Standardised practice requires using the same actions in the same ways. Standardising materials involves using the same equipment and components. Standardised information requires shared formats and mechanisms for recording and transmitting. Only with such group coordination are standards possible (Thévenot, 2009)

Social coordination can be understood as a form of infrastructure: a functional system or mechanism intended to support other kinds of functionality (Sims, 2007). Its purpose is to enable other purposes. The coordinated social collective enables standards to exist and operate, just like an electrical grid supports devices powered through electrical outlets.

Infrastructures are first enablers. They are established and persist because of what they enable. As such, they are never self-standing. Coordinated social behaviour, as a form of infrastructure, develops only in relation to what it is meant to enable. The resulting collective and its actions reflect what they are meant to support, and so they give insight into the character of the standards sought.

Infrastructures are assemblies with many diverse components (Sovacool et al., 2018) Social infrastructures, including those that enable standardisation, are also heterogeneous assemblies. Different people, practices, places, materials, objects, discourse and knowledge form part of the group's coordination and together support standards. Social infrastructures reveal the many elements of standardisation and enable a more complete understanding of standards.

Infrastructures are ubiquitous. Electrical, telecommunication and water infrastructures form constitutive parts of many social communities. They are embedded in everyday practices and the functions they enable, such as drinking tap water or sending mobile

phone messages (Star 1999). Social infrastructures are also ubiquitous. Without ongoing social coordination, basic components of life, such as language, would be impossible.

Infrastructures are important and valuable. Often, what they enable is considered necessary, such as access to drinking water. In other cases, what they enable is considered valuable, such as standardised biotechnological research. The same is true of social infrastructures. Group coordination, which comes with costs of energy and funding, gains value insofar as it enables something considered valuable. Social infrastructures suggest how advocates for standards campaign for joint behaviour.

Despite their value, infrastructures are hard to see. Some, like water piping, are physically hidden. Others, like software, have no physical existence. And many go unseen because they are overshadowed by the functionality they enable, such as motorways. Social infrastructures are even more difficult to identify. It is difficult to reveal and analyse social coordination when one is an actor involved in the group's actions. However, the social sciences routinely make crucial but opaque social behaviours visible.

Finally, like all human constructs, infrastructures can and do break. Many are designed to be stable, but continual changes over time undermine their endurance (Howe et al. 2015). Social infrastructures display the same vulnerability. Collective behaviours persist through ongoing support from the group, but all things social change over time, including the form of action necessary to sustain coordination (and its products, like standards) (Sovacool et al., 2018).

Understanding social coordination as a form of infrastructure is useful when examining standards as social constructs. First, it draws focus away from abstract ideas to what is observable. Rather than discuss units and parameters, one can investigate the ways in which practitioners realise standardisation by carrying out their work. Second, thinking about infrastructures encourages revealing things that are ubiquitous and necessary, but

also hidden and overlooked. The notion of a social infrastructure lends itself to technological, engineering thinking (a distinctive, definitive aspect of SB). Finally, social infrastructures reveal politics of social coordination.

Standardisation politics

Standardisation requires a social collective and group coordination according to specific parameters (Hanseth et al., 1996). Groups necessarily establish boundaries of membership, and if those are defined according to standardisation practices, then standards (like those planned for SB) serve as criteria for establishing who gains standing as a 'proper' member (such as a proper synthetic biologist). Their role is not simply technical. It is also about social inclusion and exclusion.

As a result, defining standards becomes a form of authority and substantial influence. If satisfying standards is a requirement to joining the field as a valid member, then those who define standards have the ability to grant and police membership. If a group cannot satisfy technical expectations, perhaps due to resource limitations, their validity as synthetic biologists is challenged. Finally, all social constructs reflect their communities. They are shaped by collective particularities such as ambitions and interests. As a result, how a group designs and carries out standardisation—how it orders its members—will reflect whatever interests are most prominent or influential. Technical decisions are shaped by the group's makeup and its commitments (Timmermans, 2015).

Conclusions and recommendations

A standardisation project is an effort to design and develop collective coordination. BioRoboost is an effort to establish social relationships and then employ those relationships to structure the field's actions into a harmonised activity. A social scientific perspective reveals these dynamics and provides insights into their operation.

Seemingly neutral or strictly technical decisions can have significant political consequences. Establishing a standard, as an act of collective coordination, is a form of organising people. Moreover, each standard demands particular behaviours. Thus, standards are mechanisms for regulating behaviour according to specific parameters. Sustaining standards requires policing that behaviour and correcting people when it becomes necessary to do so. As a result, standardisation has consequences beyond which words are used, which tools are employed and which actions are performed. Standardisation involves boundaries of inclusion, hierarchies of authority and penalties for deviation.

Recommendations for practitioners

Setting aside abstract definitions and substituting an understanding of standards as continuous social coordination has important uses for practitioners.

Synthetic biologists can recognise that standardisation problems do not necessary require technical solutions. Solving standardisation problems may be best done by adjusting how the group exists and operates (rather than developing a technical fix).

Practitioners also gain a tool to consider what social orders different solutions establish and their political impacts. Good technical solutions may be problematic socially. By examining social orders like hierarchies of authority, influence and recognition, practitioners can discard flawed assumptions about the neutrality and objectivity of 'just technical' work.

Recommendations for broader society

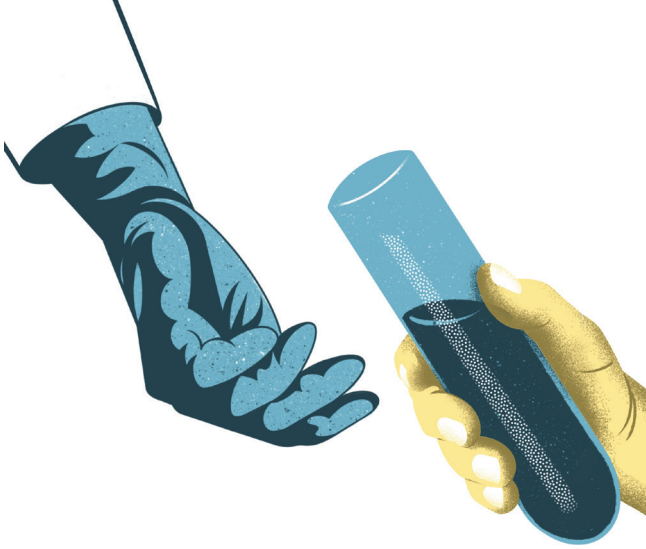
Standards are a routine part of everyday life. We are surrounded by products of standardisation. What seems self-evident is especially powerful because of its obviousness. Introducing a different perspective undermines assumptions and enables scepticism.

Moreover, technical expertise can operate as a barrier to engagement. Understanding standards as social practice makes them easier to comprehend (we all participate in social coordination). It suggests 'access points' for influence by lay actors.

Recommendation for policymakers

The work performed within the BioRoboost project has included studies of safety and risk. A social scientific understanding of standards emphasises aspects beyond the technical. It encourages policy-makers to avoid a narrow focus on technical fixes to technological risks. Solutions can be social instead.

Moreover, understanding the political consequences of standardisation can encourage critical reflection when choosing which SB projects to support with funds or policy. Questions about who gains and loses control, who is marginalised or excluded, what kinds of practices gain prominence and which are undermined all become prominent and addressable.



C9_
STRANGER COMES TO TOWN: THE HUMAN DIMENSION OF STANDARDISATION CULTURES

Overview, topic and challenge

Since its origins, the field of SB has asserted itself as a novel approach to engineering biology. Certainly, SB appeared as a new way of doing biology from a sociology of science point of view, particularly as it adopted open science visions and ambitions early on. The way in which SB presented itself as "open" covered a range of domains, from science communication to IP and open standards (McKenzie, 2013; Hilgarther, 2012). Inspired by ways of organising work in the open software culture, SB partly developed as a sort of 'redistributed biotechnology' (Delfanti, 2017). Such development revolved mainly around the idea of the "BioBrick Part" and the vision of a repository that could centralise the work (parts) of people in distant geographical locations by offering a common solution for data storage. The Registry of Standard Biological Parts was meant to not only provide 'access' to parts, but also to enable reusability of these standard parts. The idea was that parts would be widely reused and, in this way, the work within the SB community would be more efficient as researchers would not need to start to build parts every time from scratch. The reality was, though, that the level of reusability of parts in the registry was significantly low (Vilanova and Porcar, 2014).

Attempts at developing repositories of parts that facilitate reusability have been multiple ever since. A main effort towards making those repositories interoperable and to foster parts reusability is the Synthetic Biology Open Language (SBOL). As previously mentioned in the present document, SBOL is an open standard, aiming at enabling common ways of representing parts and annotating sequence data that would facilitate design (and part re-usability in new designs). Ideally, SBOL would make parts more sharable and transferable from lab to lab and from one biological contexts to another. Although many SB researchers fully support that ideal, SBOL is still not yet a widely adopted standard.

To sum up, ideals of sharing and reusability in different efforts within the SB community have not fully come to fruition, and one of the challenges within the BioRoboost project has been to understand why this is the case. The question is: how do people work in their lab in order to make their parts and data shareable and reusable? With this question guiding a round of interviews and a workshop, we have intended to move from ideas and visions to actual laboratory practices.

Progress on the topic in STS and in the context of BioRoboost

In the BioRoboost project we have attempted to build on the science and technology studies (STS) literature on open science, data sharing and reusability in biotechnology, with a discussion on the particular case of SB. In turn, we have also aimed to contributing to the field of SB by attending to actual practices in the community, by promoting reflections and open discussions across different laboratories and groups in the BioRoboost consortium and by collecting ideas on 'best practices'.

Sabine Leonelli (2016) has developed an empirical philosophy of science to describe an increasing tendency in biology to *organise the scientific work around the production of data*. Leonelli argues that biology has become 'data centric', defining 'data' as a way to 'package' knowledge so that it can travel. To conceptu-

alise data circulation and exchanges, Leonelli coined the notion of 'data journeys'. The place in which a data journey starts is often in a database. Ideally, once the data has been deposited, another researcher, in a different location, would make use of it. Data reusability becomes a sort of epistemic value in a 'data-centric biology'. Empirically, what happens often is that data does not get to be reused. The reason is, Leonelli argues, that it is actually difficult to make data meaningful in a different context of research than that in which it was produced. Thus, the real challenge to enable reusability, is to produce data that is effectively meaningful, and so useful in a different research context. A different research context would be, for instance, a different knowledge field with different research conditions, questions and scales (say, from genomics to metagenomics). There is, Leonelli notices, some people working in labs who are specialised and responsible for making data reusable. This is, *data curators*. They are key workers in a data-centric biology as they enable that data can be *interpreted* across research contexts. The possibility of (re)-interpreting data appears in this line of thought as a condition of data reusability.

Good and complete data annotations appear as a key in enabling data reusability: Researchers would like to know where and how the data was collected or produced in order to be able to make use of it. Thus, one of the main jobs of data-curators is to produce good metadata that allows other people to interpret data in other research contexts. Millerand and Bowker (2009) have noticed that the closer researchers are in terms of disciplines and research fields, the easier or less demanding data reusability becomes. A challenge is then to produce data that can travel to radically heterogenous contexts. This observation is relevant for the case of SB, to a large extent a multidisciplinary field, albeit a clear dominance of microbiology.

Leonelli, Millerand and Bowker an many of the scholars in STS, have described the kind of social, technical and legal work that is needed in other to keep data 'alive' in data infrastructures. An

iconic example of the complexity of that work is reflected in attempts at establishing standard formats for sequence data such as the GeneBank standard (Strasser, 2008). However, the case of SB presents some particularities that are worth exploring when it comes to the question of what kind of work is needed in order to make data stay and travel in digital platforms. For instance, GeneBank is a standard format that is widely used within the SB community, however, it has been argued that it is 'not enough' in the context of the kind of work that people are trying to do in this community. In so far as SB is a design-oriented field (Delgado, 2016; Delgado and Porcar, 2013; McKenzie, 2010), it requires standard formats that can capture not just structural information on sequences, but also functional information: what can different genetic elements *do* when assemble together in a certain design? SBOL originated to capture in a standard that kind of information, assuming that it was that kind of information what would make parts travel more easily, thus enhancing reusability.

A vision that has been a driver in SB is that of a community where standard parts can be easily exchanged and used in different contexts and by different groups, and where the field is developed out of collaborative and redistributed work across laboratories. The realisation of that ideal depended on people sharing standards that would enable parts circulation. Yet agreeing on those standards has proven to be more difficult than initially thought. During the BioRoboost project, and investigation was carried out on why existing standards may present shortcoming to 'capture' the information that is needed in order to make parts reusability happen. This investigation was based on interviews with PIs, researchers and post-docs on reusability practices in different labs (specifically, 18 qualitative in depth semi-structured interviews lasting 25-80 minutes were performed), as well as a "Best-practices" workshop.

During the interviews, informants referred to experimental data (practices for reporting experiments) and sequence data (practices for annotating data). The round of interviews revealed that

researchers in the different labs struggled with different kinds of complexity and contexts dependencies when trying to 'pack' their data in standard ways, and so to make their knowledge shareable. They referred to biological, social and technical contextual information that was difficult to capture.

Biological contexts. The three emerging topics were: (1) *complex systemic relations*, order and architectures (functionality of parts may change depending on order); 2) *emergency and indeterminacy* (including intrinsic uncertainty); and 3) *capacity of living systems, flexibility and evolution*. As one of the PIs in the BioRoboost Consortium expressed it "We take this as an essential part of a biological part: that is has flexibility built in, due to the fact that it needs to evolve further".

Social contexts. Emerging topics under this theme were: (1) need to adapt data annotation to particular *contexts of reception* (including particular 'publics' and 'technical contexts') and to adapt data characterisation and annotation to particular contexts of research with particular research questions and goals; (2) good reporting appeared as a matter of *personal skills, experience and training*; (3) much experimental knowledge was described as being *experience-based, situated and tacit*, and therefore hard to capture and encode in standards; (4) data sharing standard formats are not enough and important information still shared through *informal channels*; and (5) *freedom, flexibility and creativity* was claimed to be a necessary condition of scientific knowledge (science is discovery-driven rather than data-driven?).

Technical contexts. An issue that was mentioned in this regard was that equipment is used differently in different laboratories, and measurements may vary. Measurements are likely to change as laboratory equipment is updated and tools such as gene sequencers or imaging technologies evolve. Finally, not all labs have access or need the same kind of equipment.

Conclusions and recommendations

A first reflection to draw out of this round of interviews and, in contrast to Leonelli's image of a 'data-centric biology', beyond data 'interpretation', experience and material dimensions (such as lab equipment) matter when trying to make data reusable. Informants expressed in different ways a 'feeling for the experiment', in the sense that the relation that researchers have to their experiments is personal and unique. Taken together, the emerging topics presented above suggest that SB is perhaps more of an 'experimental culture' than other more 'big-data' oriented fields such as metagenomics or other versions of systems-biology. Related to the latter, in SB it may be more difficult than in other biotechnology fields to establish a clear cut between replicability and reusability as the experiential and material conditions of experiments are necessary in order to make actual use of parts (differing from big-data driven research).

Finally, there seems to be a consensus in the BioRoboost consortium that for replicability and data reusability, standards are necessary. However, the community of SB is heterogenous and research projects, objects and objectives vary (despite a majority number of microbiology labs). If standards always respond to the need of a community, it would be necessary to identify first the community and the need.

C10_
BIOSAFETY

Overview

In 2015, the European Commission's SCENIHR presented a series of opinions on how to close major gaps that had been identified in an earlier SCENIHR study, with respect to performing reliable risk assessment for SB (Scher et al., 2015). This included recommendations regarding the need to develop tools for identifying emergent biological properties and safety issues in SB products, and encouraging the use of GMO comparators. Further recommendations were aimed at improving the understanding at the mechanistic level of the underlying principles of biological containment and survival after escape (including the need for metrics of containment, particularly in relation to the question "how far" in semantic containment), and increasing awareness and compliance of citizen scientists (including those self-identified do-it-self biologists, aka DIY-Bio) with national biosafety rules.

One of the objectives in the BioRoboost project was to address, based on the SCENIHR publication, to which extent standardisation in SB would contribute to improve or hamper its risk assessment and biosafety. This challenges was tackled with a dedicated three-day workshop on biosafety in SB, 20 stakeholder interviews, and the curation of a one-stop-shop biosafety database for researchers, industry, regulators and other stakeholders.

Progress

A workshop titled *"Towards enhanced biosafety and risk assessment standards in synthetic biology"* was held on the 7-9 October 2019 at the EMBO offices in Heidelberg, Germany. The 17 workshop participants included representatives with interests and expertise in academic and industrial research, standards, and risk assessment. They were biosafety experts, practitioners of DIY biology, publishers, researchers and industrial leaders carrying out and supporting the research itself.

The major findings from this Workshop were:

- For the industrial stakeholders represented at the workshop, standards generally, and especially those for risk assessment, are seen as important to work more effectively and to have a clear understanding about industry benchmarks and supporting platforms.
- For regulators, the importance of case-by-case evaluations should be emphasised, and it is critical is to understand in which ways standards could enable and support that individual assessment, and how to avoid putting different cases in the same category. This is true for biosafety organisations as well.
- The participants from the research field pointed out that arrays of experiments and methods that would help standards to be implemented have not been defined or carried out, or in cases where they have, the results may not be available. In most cases researchers have no or little incentive to make standards for these experiments as they are usually not considered an inherent part of their own research and thus are not resourced properly, and rarely lead to publications.
- The representatives of the larger research community, including biohackers and citizen science, noted with concern the strict regulations in Europe and would like to see a more liberal framework to carry out experiments.

- Publishers can in fact already leverage some changes, namely, to require a description of standards used when publishing a paper. They would only do this if there was a clear interest from the research community to do so. They also pointed out that such requests cannot be too burdensome; otherwise, the researchers would eventually seek out journals with less onerous requirements.
- Furthermore, the Standards organisation clarified in the context of all of these interventions that their role is primarily to organize and manage the process of deliberation when developing a Standard on a national and later international level.

From the expert interviews (from June to September 2020), opinions of 20 interviewees from industry, research, regulation, policy, and standardisation organisations were analysed, aiming to identify opportunities and challenges for developing biosafety standards for SB. The interviewed experts, in general, thought that biosafety is important for SB, and that more biosafety standards need to be developed. The safety-by-design principle was seen as helpful for enhancing biosafety in research and development. Since SB is an interdisciplinary research field with broad international cooperation, an international scope for biosafety standards was predominantly seen as the goal, although a national regional, or even European level could also make sense. The following expert views were elicited:

- *Importance to integrate biosafety in SB standards:* generally considered as important but overlooked. It is important to follow SB standards (if any), and equally important for biosafety to be developed alongside with other standards. There are differences between "need for standards" vs. "need for interoperability".
- *Opinions on Safety by Design (SbD) for biosafety:* agreed that SbD would help to enhance biosafety; it would be a good example of implementing Responsible Research & Innovation (RRI), a central part of engineering science and a good

starting point for experimental design. The concept has been worked extensively and in some places the concept has already been implemented into their real-world practices.

- *Benefits of biosafety standards:* making it easier to gain public acceptance and providing clear guidance to conduct research. It would be interesting accelerate tech transfer from contained use to (semi) open applications, allowing more people to conduct research if biosafety loopholes have been secured.
- *Disadvantages of biosafety standards:* standards might add more workload and intricacies to everyday SB research and development, in particular by adding more constraints and decreasing the efficiency of conducting research and development. The lack of clear frameworks, definitions and measurable features to draft the standards may exacerbate these.
- *Impacts on research community:* overall positive, the research community would have rules to follow, gaining more confidence if there were standards to follow.
- *Impacts on industry:* might provide clarity on regulatory process and make communications with the public and regulatory authorities easier, while also having to deal with additional cost, trade-off, and regulatory burden.
- *Impacts on regulatory authority:* might help to gain public trust but regulators might not have the capacity themselves to develop the standards.
- *Usefulness of standards on risk assessment on cross-category types of research:* biosafety features found in one organism in one condition would not necessarily be valid in other settings: how can standards set for a known condition fit for an unknown condition? Some suggested that "deep" engineering on microbes should not be done at the first place.

For a one-stop-shop biosafety database it is important to have a comprehensive overview of the relevant data and information on biosafety. Therefore, this information was retrieved from peer

reviewed articles, well-established websites, and relevant documents from regulatory bodies. From the literature mining, different existing biosafety relevant standards have been reviewed, such as different containment options (physical, chemical, biological), and a few areas in the field of biological containment where standards would be beneficial but are not yet available have been reviewed. The initiatives in SB that have attempted to establish standards have been reviewed, such as iGEM and the BioBricks Foundation parts (for genetic circuits), SBOL (for in silico design), SEVA (for plasmids), MoClo (for cloning), BioXP (for DNA assembling), DNA-BOT (for automation), the existing ISO and CEN standards (for biotechnology and biological risk managements), as well as proposed chassis organisms for standardisation and REACH standards for chemical safety.

Furthermore, a comprehensive analysis of existing biological containment strategies has been performed, covering the description of the strategy, its feature(s), whether it has been already tested or proposed, measured or calculated escape frequency, tested or proposed applications, and other possible concerns.

In total, eight categories of containment strategies have been identified and reviewed: physical/chemical containment, auxotrophy (synthetic or metabolic), kill switch circuits, somatic containments, multiple layer containments, safeguard CRISPR strategies, de novo genome synthesis approaches, and chromosome free systems (see Table 3 for some examples).

Table 3. Containment strategies.

	Underlying principle	Example
Physical approach	Physical systems for confinement within a defined space (i.e. labs, bioreactors) and conditions (i.e. air filter, temperature, pressure, light)	Labs, bioreactors, UV light, heat
Chemical approach	Chemical agents that can limit growth or kill microbes	Disinfectants, antibiotics

Auxotrophy	The growth of the microbes depends on the supplementation of certain chemicals, thus the survival of the microbes outside the containment environment is limited.	Thymine dependent *L. lactis* (Steidler et al., 2003), benothiazole dependent *E. coli* (Lopez and Anderson, 2015)
Kill switch	The survival of the microbes depends on the synergy of the toxin and anti-toxin pair, thus the activation of the kill mechanism only will lead to cell death.	Conditional suicide system using *sacB* in *E. coli* (Recorbet et al., 1993)
Somatic containment	The genetic information flow has been confined by harnessing a different set of genetic codes and/or genetic information storage systems.	Genome recoded *E. coli* (Chatterjee et al., 2014), Semi-synthetic *E. coli* with a unnatural base pair (Malyshev et al., 2014)
Safeguard CRISPR	Using CRISPR-Cas9 inhibition molecules to control the genome editing activities of the system	Gene drive inhibitor by AcrIIA2 and AcrIIA4 in *S. cerevisiae* (Basgall et al., 2018)
Multiple layer	Combining several containment approaches for maximum confinement.	GeneGuard in *E. coli* (Wright et al., 2015), CAMEOS in *E. coli* (Blazejewski et al., 2019)
De novo genome synthesis	Engineering microbes by novel genome sequences either by adding unique DNA sequence as barcode, or with recoded sequence	Version Control System in *E. coli* (Tellechea-Luzardo et al., 2020)
Chromosome free systems	Removing the native chromosomes from the cells by digestion of heterogonous nuclease and then supplementing with engineered genetic circuits to synthesise molecules of interest	SimCell of *E. coli, Pseudomonas putida, Ralstonia eutropha* (Fan et al., 2020)

Challenges for biosafety standardisation

During the Workshop, the participants raised several challenges, particularly the lack of incentives for most if not all stakeholders to actively engage in the development of standards, and the difficulty to create standards that are useful but do not add additional burden to their users.

From the expert interviews, the following challenges were identified: the need to gain more knowledge about ways to improve biosafety and establish safety metrics, to ensure that these standards do in fact facilitate risk assessment, and to make sure that the implementation of biosafety standards are as much as possible not a burden to those working in research and development. Biosafety is a broad and comprehensive goal that goes beyond mere technical solutions or standards that entail the continuous engagement of stakeholders and fostering a culture of safety.

From the literature mining on biosafety containment strategies, the major challenge identified is that metrics are mostly unavailable but needed to set standards for biological containment strategies.

Conclusions and recommendations

Biosafety is thought to be important for SB and there is a need to develop biosafety standards. The safety-by-design principle is helpful to enhancing research and innovation. Since SB is an interdisciplinary research field with broad international cooperation, an international standard format of biosafety standards would be favoured, although a regional level or even European level of standard could be also developed.

C11_ BIOSECURITY[3]

Implications of standards in biosecurity

The issue of biological standardisation in relation with biosecurity has not previously been addressed in detail. In this report, we identify a series of aspects linked to standardisation and their implications in biosecurity (Figure 4).

Universality

An example of an almost-universal device is that of smartphones. There are millions of them on Earth and in many countries most of the citizens have one (or several). Smartphones are standard in the sense that, despite the existence of different models (or strains/species, in the analogy in biology), they work in an equivalent way. Receiving or sending a WhatsApp message, for example, is largely independent of the smartphone used, because they all work alike with that app. It is pretty obvious that an informatic virus, a particular fake news or a geolocation involving smartphones could have an effect on all of them. In other words, the universality of a device is linked to the universality of the risk.

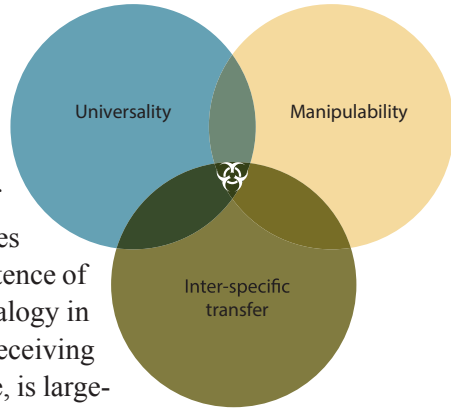


Figure 3.
Aspects of biological standardisation as they relate to biosecurity.

3 This section is an adapted version of Trump et al., 2021.

Not unlike smartphones, making a standardised platform for SB would universalise the risk. If a given plasmid, virus or cellular chassis was made universally available, so would be the risk derived from a malicious use.

Chassis and Trojan horses

In the Homer's Iliad, Greek soldiers entered the city of Troy hidden in a wooden horse. It has to be stressed that the horse was not the weapon, but the vehicle of the actual weapon (the army). Considerable effort was required to set in place the horse as a chassis of the weapon, but once in place, its further use because much easier (although there are no mentions in the Iliad of a further use of the horse). In the example above, smartphones were described as standard devices that may serve as chassis/Trojan horses. Biological chassis, provided that they were robust, easy to maintain and to amenable to modification, could also be considered as biological Trojan horses: inoffensive by themselves, but susceptible of being used to deliver bioterrorist actions because of their manipulability.

Breaking down the species barrier.

As we have stressed in the previous section, several currently ongoing efforts are successfully allowing microbial transformation by introducing plasmids in a range of different species (see the description of SEVA plasmids above). The obvious implication in terms of biosecurity is that pathogenic DNA fragments could be inserted into harmless bacteria turning them pathogenic or, alternatively, pathogenic bacteria could be turned into more lethal agents by including certain biological circuits from taxonomically distant bacterial species.

Standards as social constructs.

As a final remark, we strongly believe that it is important to be aware of a common misconception of the "inner" nature of standards. Robustness, reliability or ease of use are highly relevant

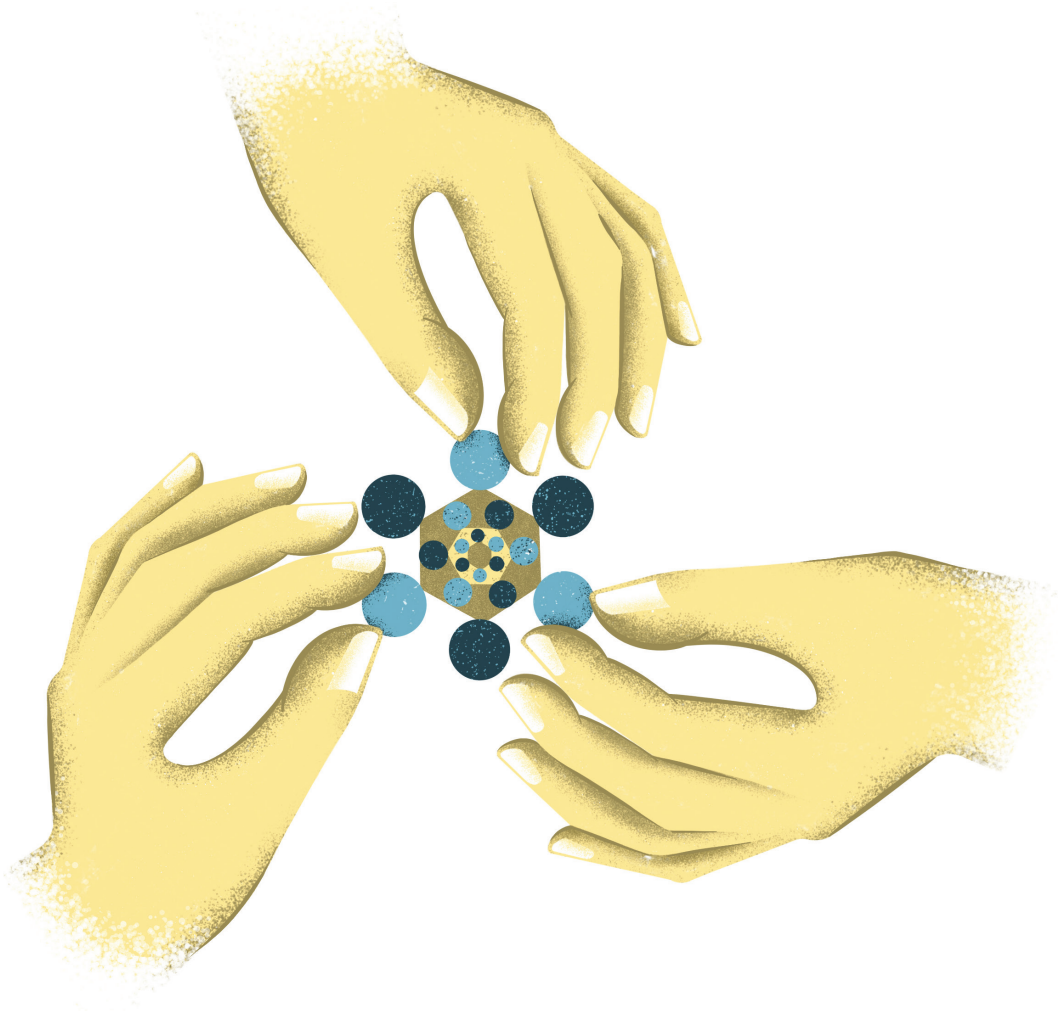
features of any standard. That said, though, a standard must be acknowledged within a group of individuals on a basically arbitrary basis (see metric units, flags colours, and any other "convention" standards). This has implications in terms of biosecurity assessment, since discussions tend to focus on the risk of biological parts *per se*, and not on the risk of standards because they are standards. In other words, the value of a standard does not only rely on its inherent properties but largely depends on a subjective, coordinated social decision to elevate a particular biological part/system/device to the category of a standard.

Conclusions

As we have discussed in this section, standardisation in biology is a complex, still in process, path that will be central for SB to fully develop its potential. Standardisation could finally make SB's promise come true and make biology easier to engineer. As we have described above, and in terms of biosecurity, this fact will ineluctably be linked to an increased risk of the discipline because of the universality of the biological systems (and actors), their amenability as Trojan horses, and the possibility of (more) easily breaking the species barrier. The question arising here is not thus whether advances in standardisation will be linked to increase bioterrorism concerns, but to which extent the risk is proportional to the standardisation level accomplished. However, and when it comes to biosafety, the situation is totally different. SB has an already-not-that-short background of perfect biosafety, even if the discipline has been working without standards for most of its historical existence. The universalisation of biological parts, circuits, chassis cells, and procedures can only be associated with further increases in safety for both SB practitioners and the environment. Interestingly, and even in the cases in which standardisation will take time or will not happen at all, biosafety control mechanisms (in the shape of standard safety protocols and guidelines) will ensure the minimisation of risks.

As a general conclusion, the standardisation of SB is a complex path, most of which is still in its infancy. The success of this process will result in immense economic and societal benefits. The risks of SB in terms of biosecurity are only partially known, and the implications of the possible success of the ongoing standardisation process in the biosecurity threads of this emerging discipline deserve further study. By contrast, the benefits of standardisation to increase biosafety are obvious. In fact, both aspects (increased biosecurity risk, decreased biosafety risks) are two sides of the same token: standardisation will contribute to make a reality SB's classical expectation of making life easier to engineer. This could lead to more incidents of deliberate misuse, but that will pale compared to the decrease of unintentional hazards.

PART 4
FINAL CONCLUSIONS, FINAL RECOMMENDATIONS AND BEYOND



Standards are not what most people think they are. Standards are not universal, perfectly fit parts or procedures, the inherent quality of which makes their adoption as universal, exchangeable units, unavoidable. As we have seen in this book, standards are social constructs, which means that they are rules, codes, parts or techniques that have been acknowledged by a group of individuals as such, and that have been elevated to the status of reference parts because of that agreement. Obviously, it is highly desirable that a standard is simple, easy to produce and replicate, behaves in the most robust way and yields consistent results. All those positive features will certainly contribute to increase the possibilities of the standard being widely adopted. But the social consensus of what a standard is, is a sine qua non condition.

There is no need to describe here to which extent standards have favoured the emergence of our modern civilisation. Their use in all engineering disciplines is simply imperative. This does not mean that standards are always the best choice, since the link with flexibility and innovation is often disputed. Interestingly, there are similarities between the pros and cons of standards on innovation, with those of patents. In both cases (of patents and standards), their potential is beyond doubt, but they may not necessarily be the best option in all circumstances.

As a conclusion of the EU-funded project BioRoboost, in the frame of which this white book has been written, we advocate standards as the brokers of a new type of Biotechnology which moves quickly and responsibly from laboratory experiments to large-scale processes, and we argue that early involvement of the public, amateur biologists and other stakeholders will be central

to steer the direction of SB towards a technical, social and environmental success. In order to reach that goal, it is necessary to overcome national and political barriers and to gather key players in a permanent forum, in a dynamic way. The key to success will be the at the interphase of technical and scientific soundness with legal requirements and consensus among end users.

Technically speaking, standardisation in SB is a very complex process. One of the key points is metrology, that is, the scientific study of measurement. Unfortunately, SB as a field has not progressed much since the first efforts on the topic started. The main difficulties for consolidating metrology in SB include, of course, the technical complexity; but the reward system in academic research does not help: improving standards and metrology is a low priority both for high impact journals as well as funding panels and reviewers. Even where progress has been made, adoption by the community has been low. This leads us, again, to the bottleneck of reaching consensus by the community of SB practitioners and other keyplayers, including journal editors, whose role is defining formats (and thus, standards) of the articles they publish could boost standardisation and metrology in SB.

Most of the advances in SB have been carried out in microorganisms; in most cases, in bacteria, and the most common species has been, of course, *E. coli*. But SB is more than biological engineering of the bacterial workhorse *E. coli*. If real-life challenges, particularly environmental ones, are to be tackled with SB approaches, alternative microbial chassis resistant to desiccation, able to form symbiosis with plants, able to biodegrade a wide range of pollutants or with a large thermal stability are needed. The first systematic proposal towards defining a reduced range of microbial chassis alternative -and complementary- to *E. coli* was the proposal of the BioRoboost project. Since then, several authors and reports have supported this view.

If there is still a long way to go to have optimised bacterial chassis, the standardisation of other biological systems is still in its infancy, but significant achievements have recently been made.

In yeast, for example, the studies aiming to develop toolkits and DNA assembly methods for various yeast species have greatly contributed to the standardisation of the related DNA parts and methods. This has allowed bakers' yeast, *S. cerevisiae*, to become an important SB chassis, in addition, to allow emerging new yeast species to benefit from the previous standardisation knowledge acquired, in a process elegantly parallel to the bacterial chassis development mentioned above.

In eukaryotic systems, particularly mammal cell lines, the immaturity and importance of the field has moved us to make two main recommendation: to identify mammalian cells as preferred chassis in specific application scenarios and to foster the adoption of part/assembly standards through a combination of incentives and imperatives (i.e. journals mandate compliance with specific standards); and to engage with the Global Biofoundry Alliance to promote mMoClo/EMMA as MSB "assembly standards" in their operations. Again, as it was the case for bacterial and yeast systems, the development of a "pantone" of alternative chassis within each system, along with the need of consensus among practitioners are our main suggestions.

After the fiasco in terms of communication of GMOs, voices have raised to emphasize that lessons are to be learnt for SB. But an obsession regarding public perception of SB would be a mistake. In line with Responsible Research and Innovation practices, a holistic strategy to foster standardisation in SB must include all keyplayers -and the public- already for the beginning of the process (that is: now). The objective is to carry out a co-creation assessment of the risks and benefits of the technical advances. This includes an honest and reflexive description of environmental, biosafety and biosecurity concerns.

Biosafety is obviously important for SB and we highlight the need to develop biosafety standards. The safety-by-design principle is helpful to enhancing research and innovation. An international standard format of biosafety standards would be favoured, although a regional level or even European level of standard could

be also developed. Interestingly, fostering standardisation in SB would certainly lead to decreased biosafety concerns, since the very idea of standards -reproducibility, universality, robustness- is at odds with that of accidental event. Biosecurity is a totally different case. The risks of SB in terms of biosecurity are only partially known, and the implications of the possible success of the ongoing standardisation process in the biosecurity threads of this emerging discipline deserve further study. It seems reasonable to expect that fostering standardisation in SB will contribute to reach its famous goal of "making biology easier to engineer". It would thus be cynical not to expect that malicious use of biological systems will benefit from standardisation in biological systems, simply because it will be easier to carry out deep genetic modifications. In summary, consolidation of standardisation in SB will indeed lead to a significant decrease of unintentional hazards, but could also promote more incidents of deliberate misuse. That said, it is expected that natural pathogens, and not SB-issued ones, will be the main concerns in terms of both biosecurity and biosafety reasons at least for the next few years.

To sum up, SB will benefit from the standardisation of biological parts, measurements, procedures and from the development of a defined and small number of biological chassis that will ease the industrial translation of the scientific developments. As we have emphasized before, standards in biology will only be a reality from a crosstalk among all keyplayers involved. This book includes many specific recommendations for policy makers to foster standardisation in SB, which might in fact be condensed in a very simple one: promote consensus.

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