Fast biofoundries: coping with the challenges of biomanufacturing

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Abstract

Biofoundries are highly automated facilities that enable the rapid and efficient design, build, test and learn cycle of biomanufacturing and engineering biology, which is applicable to both research and industrial production. However, developing a biofoundry platform can be expensive and time-consuming. A biofoundry should grow organically, starting from a basic platform but with a vision for automation, equipment interoperability and efficiency. By thinking about strategies early in the process through process planning, simulation and optimization, bottlenecks can be identified and resolved. Here we provide a survey of technological solutions in biofoundries, their advantages and

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limitations. We explore possible pathways towards the creation of a functional, early-phase biofoundry, and strategies towards long-term sustainability.

Highlights.

- Laboratory automation is playing a crucial role in the development of synthetic biology methodologies, allowing fast and inexpensive engineering of genetic circuits for a wide range of biotechnological applications.
- Synthetic biology laboratories are in the midst of a paradigm shift, catalysed by biofoundries and their new approach to engineering biology.
- The innovative, resource-intensive and risky nature of biofoundries demand informed decisions before committing to building a new facility.

Biofoundries are changing how labs engineer biology.

Synthetic biology is an interdisciplinary field that combines biological, engineering and computational principles to create complex biosystems [1,2]. To do this, scientists relied until recently on bibliographic research and previous knowledge to design and build constructs, essentially one by one. This process is time consuming and expensive and can be compared to the early mechanical engineering steps where the production chain was almost completely manual [1]. However, as with ancient handcrafts, scientific advancements are allowing the field to rapidly evolve. A key is **laboratory automation** (see Glossary) that tries to achieve higher levels of reproducibility, a feat that synthetic biology has been pursuing since its early stages [3]. Automation opens up the possibility of establishing high-throughput synthetic biology, similar to modern engineering technologies, where thousands of biosystems can be potentially built in short periods of time. Automation allows error free protocols and 24/7 workflows. The throughput that is enabled allows broad exploration of the design space and production of large variant libraries with the generation of large data sets that can be used for future modelling and redesign.

Biofoundries are the biotechnological entities that can move forward this research area. Such a new approach changes the paradigm of molecular biology laboratories towards laboratory automation, data acquisition and integration and the use of mathematical models and **artificial intelligence** (AI) technologies to tackle all the steps in the **Design-Build-Test-Learn** (DBTL) cycle. The idiosyncrasy of the biofoundries perfectly fit into the DBTL cycle and complement it in several ways. The biofoundry life cycle (see Key Figure) described in this work, showcases the main possible tools, methods and processes driving the iterations of the DBTL cycle.

The interest in biofoundries as disruptive entities in the bioengineering field has been highlighted by the creation in 2019 of a Global Alliance of Biofoundries (GBA) to coordinate their activity around the world [4]. However, even though biofoundries appear as an essential actor for the long term development of an engineering-like synthetic biology, they do not come without limitations and setbacks, especially for newcomers. A detailed description of general considerations for research groups interested in establishing a new biofoundry can be found in [5].

Here, we present a set of possible approaches to improve the current risk-benefit ratio of setting up a biofoundry. We coin the concept of early-stage biofoundries and we propose different paths towards building next-generation "fast biofoundries".

Early planification, the key.

The cost of establishing a biofoundry requires a long-term business model for biofoundry sustainability that should be initially implemented considering both its potential users as well as target market [5]. To that end, strategic analyses such as SWOT (strengths, weaknesses, opportunities and threats), PESTLE (Political, Economic, Social, Technological, Legal and Environmental factors), and stakeholder analysis (Tables 1, 2 and 3 respectively) should provide a better understanding of such a business model. In order to avoid market saturation, it is essential that biofoundries find a trade-off between flexibility and specialisation into one particular niche of the synthetic biology market.

As more automated laboratories are being established worldwide, higher **standardisation**, provenance tracking, traceability and reproducibility among labs are achieved [6]. A business model of biofoundry as a service has become possible, where each facility can specialise in some of the tasks on the Design-Build-Test-Learn cycle rather than developing a fully automated engineering platform. In such a scenario, a brokerage system should be put into place so that the different complementary steps can be strategically outsourced to different labs. To that end, the adoption of standards like the OpenMTA [7] by biofoundries for exchange of plasmids, strains, samples and reagents would greatly promote collaborative research and joint efforts. Furthermore, technoeconomic assessments and life cycle analysis should be implemented to evaluate the technical and economic viability of the biofoundry, as well as the environmental impacts associated with all stages of the product's life [8,9].

Finally, a good planification assessment for a nascent biofoundry is the description of a business plan that among other things clearly defines the goals of the biofoundry, identifies all the key players for its success and determines its incomes and outcomes of capital. Biofoundries (either

completely private or embedded in a public institution) need to endure, cover their costs and recover the investment after a period of time. For that reason, we argue that a **business canvas model** is a valid tool for biofoundries, even if their business model might not necessarily be focused on profit-making plans.

To assist in this planning, we present in Figure 1 a suggested business model canvas designed for a generic biofoundry.

Biofoundries to address global challenges.

Next, we define two key example areas of research that can benefit from the methodologies used in a biofoundry, whereas additional diversification paths that any biofoundry could take to supplement their activity are discussed in Box 1.

Biofoundries for biomanufacturing.

Metabolic engineering uses synthetic biology tools and concepts to optimise the synthesis of a target compound, from single-cell to large-scale fermentations [10]. Synthetic biology and metabolic engineering underpin modern **biomanufacturing**. Bioproduction usually relies on the expression of metabolic pathways in host microorganisms in large scale fermentors and in the purification of the target compound. However, the initial phases of development require molecular biology techniques to establish the best producer candidates before scaling up. This stage requires combinatorial DNA part assembly, it is time consuming and, thus, an excellent candidate for automation. Notably, bioproduction has been at the forefront of the biofoundries' interests since the beginning. Some recent examples of biomanufacturing biofoundries include the SYNBIOCHEM [11], the Agile [12] and the MIT Broad biofoundries [13]. In 2016, as a result of the pressure test administered by the U.S Defense Advanced Research Projects (DARPA) to assess the rapid response capacity of biofoundries, the MIT-Broad Institute Foundry and academic partners achieved initial production systems for 6 out of the 10 target molecules requested, in the given time limit of 90 days. Later, the SYNBIOCHEM biofoundry in Manchester accomplished the production, from scratch, of 17 from 25 materials monomer targets in 85 days [11]. These tour de force examples illustrate how automation helps make synbio faster, motivating the field to formalize this high-throughput and the integration in biofoundry platforms. A recent review including these and other successful examples of biofoundries applying strain rapid prototyping for biomanufacturing can be found in [14].

Biofoundries for genetic circuit engineering

The implementation of genetic circuits to dynamically control gene expression is at the core of many synthetic biology applications. Such circuits are regulatory networks that turn inputs into outputs according to genetically-encoded rules—a genetic *program*. Although current circuits [15–17] are effective and predictable, there is ample room for improving their information-processing capabilities by engineering increasingly complex and more robust devices [18]. To this end, a more rigorous, while easy to scale-up, implementation process is needed. Within this context, biofoundries can make a difference, allowing researchers to [i] better characterise the dynamic features of biological parts and systems, and [ii] robustly build libraries for different genetic programs.

Biofoundries can be used to automate the implementation of well-characterised libraries of genetic parts [19] at relatively little cost. For example, allowing large-scale characterisations of genetic noise. Since part performance is often far from digital (e.g. promoters with pure on/off behaviour), the success of a biological program (even simple combinatorial logic gates) is often determined by small noise patterns in, for instance, a regulatory protein [20] or an expression product [21]. Characterising one part at a time is not only time-consuming, but impractical. Automation can help building and testing sequence libraries, thus providing an overview of the parameter space of single circuit nodes. While manual steps are error-prone and difficult to compare against each other, automated implementation promises robust and standardised [22] building processes.

The development of synthetic biology *workflows* [23,24] and design automation pipelines [25] is complementary to the establishment of biofoundries. Both efforts converge in, for instance, bridging the gap between in-silico modelling and in-vivo testing; enabling model-based implementations that will increase the accuracy of circuit behaviour. Considering circuit inputs (i.e. with which the genetic circuit is regulated), biofoundries offer the potential test features such as gradients and environmental conditions—via liquid handling robots—informed by previous in-silico models. As for the algorithmic performance of the circuit, different measuring techniques will allow for a more detailed dynamic characterisation. For instance, a biofoundry setup with bioreactor modules for continuous culture can test evolutionary dynamics, which will ultimately inform mathematical models for a better biodesign. We argue that the coupling of models and experiments—let alone data science—will be greatly enhanced by the use of automation facilities.

The catalogue of techniques for measuring circuit output can be expanded—beyond the use of fluorescence proteins—targeting specific case-studies. For instance, in order to measure stochastic perturbations in transcriptional networks (e.g. for analogue computing [26]), the automation

pipeline should contain machinery for RNAseq data (or even Ribo-Seq units [27]). Other applications may require the characterisation of cells under the microscope via microfluidic devices—even the use of optical tweezers may help gather the correct data for the biological process at stake.

A biofoundry's starting package.

Basic equipment.

The cost of the equipment required to set up any synthetic biology laboratory and especially the Build and Test divisions of a biofoundry can add up to five or even six figure budgets in euros. This can be a hindrance that may lead teams to give up their interest and transition into more affordable projects. A trade-off between abandoning altogether the conversion of a laboratory into a biofoundry facility and investing a substantial spending effort may be to start small and grow at a slow pace towards a fully automated and integrated laboratory [5]. This, coupled with collaborative work with different laboratories with different expertises and equipment availability, distributes the workload of the biofoundry, easing the transition to an optimal setup. In addition, we argue that, at least in the early phases of development, currently available options allow researchers to set up functional semi-automated laboratories with limited resources that may act as a spearhead for further funding. These affordable options are arguably faster to fund and obtain than more expensive alternatives for most labs and can provide feedback on the needs of the biofoundry before committing to more expensive equipment alternatives. Another important factor to take into account is the adaptability required to incorporate biofoundry principles into a traditional laboratory, in particular, the upgrade of essential laboratory equipment needed to transition into automation. For instance, the new setup for a biofoundry is going to need higher throughput in basic tasks like PCR and centrifugation. Acquiring one or more 96-well thermal cyclers and plate swing bucket centrifuges can hugely decrease the time to production and increase the modularity in the biofoundry.

Apart from the more traditional options of renting and second-hand purchasing, affordable and Do It Yourself (DIY) equipment and open-source hardware are becoming more present in the synbio laboratories. Next we describe some equipment alternatives and some considerations to bear in mind before acquiring or building one.

Quite likely, the first major piece of equipment of any biofoundry (apart from essential devices in a molecular biology laboratory like freezers, centrifuges, incubators and thermocyclers) would be an **automated liquid handler** [5]. The community has developed open-source alternatives for liquid

handlers like EvoBot [28] and OpenLH [29]. When looking for affordable commercial options, the Opentrons robot stands out. Starting at 5000$, this open-source liquid handler can meet the requirements of an early biofoundry and since it can be programmatically controlled using custom made scripts it is suited for the data integration required for this kind of laboratories as previously described. Opentron robotic platforms also allow a good level of customisation and adaptation to different purposes, something needed in biofoundries when not all the pieces of equipment required for a project can be afforded. This has been highlighted in [30], where the authors managed to adapt an Opentrons OT-1 to pippet nanoliters of samples. In 2020, Storch and colleagues [31] described the adaptation of Opentron equipment to the low-cost assembly of genetic parts (ranging at a cost of $1.50–$5.50 per construct), something fundamental for any synthetic biology project. After the assembly reaction, the circuit in a plasmid needs to be transformed, usually in *E. coli*, to be propagated. However, the transformation procedure is a bottleneck for automation since it requires heat shock or electroporation treatments to be added to the robotic pipeline. To solve this, in [32] it was described the automation of the cloning and transformation protocols using natural transformation capabilities of *Acinetobacter bayly* using an Opentrons robot.

Currently, the market offers a wide range of liquid handlers options available in the mid or top-range pricing options like the Hamilton's STAR, Eppendor's EpiMotion or the Tecan's Evo. Newer technologies, away from the traditional liquid dispensing based on pipette tips include the Labcyte's Echo and iDOT alternatives.

After constructing the desired circuit, the usual next step is transformation in a plasmid-propagating strain. Researchers need to pick transformant colonies and test them for the presence of the target genes and phenotype. This tedious task can be automated using colony-pickers. Low-cost commercial options for this piece of equipment are generally still not available, however there are alternatives to consider. For instance, in 2019 the Marburg iGEM (2019.igem.org/Team:Marburg/) turned the OT-2 into a colony picker by adding a RaspberryPi and a camera to an Opentrons robot to identify colonies in the agar plate. The robot then lowers the pipette tip into the colony coordinate and transfers it to another location. In addition to this addon to a commercial liquid handler, in 2018 [33] researchers described the construction of a colony picking robot that, instead of sterile tips, heats a needle at 400°C to sterilise it and continue the picking operation at an estimated rate of 2400 colonies/hr, however no details of the total costs of construction are given.

It is always important to check the results of the genetic engineering process via sequencing. For small-scale engineering experiments like cloning reactions, the go-to option of most laboratories is an outsourced Sanger sequencing service. However, when the number of samples to be sequenced is

high or the engineering process may have involved genomic changes, NGS can be a better alternative. If the laboratory wants to sequence their own strains or plasmids in-house, Nanopore DNA sequencing devices like MinIon offer affordable, quick, high throughput results [34].

The next step in a standard experimental workflow, would likely be culturing the colonies in liquid media and obtaining early and quick results like growth profiles, fluorescence emission or other types of measurable outputs from the engineered strains. Plate readers are the instrument of choice in this case. However, more affordable alternatives are called for. To this end, in 2019 Karol and coworkers [35] presented an open-source plate reader with absorbance, fluorescence emission reading and optogenetic capabilities. The instrument can be built for $3500 and can be programmatically controlled through Python.

Other experiments require deeper understanding and control of the media and growth profile for longer periods of time and mimic early scale-up conditions before proceeding to more industrial settings. Bioreactors are the instruments of choice in this case. Nevertheless, the prices of traditional commercial bench-top bioreactors are particularly high. In recent years several studies have shown the possibility of building homemade bioreactors. In [36], researchers built a reactor at ~700€ per four-chemostat module. It allows several types of experiment to be run at the same time and the size of the vessel (a commonly used laboratory bottle) allows it to run replicates in parallel. A highly customizable DIY framework for automated cell growth experiments was presented in [37]. eVOLVER, was specially designed with the high-throughput required in modern laboratories in mind. The article described the evolution study of yeast in 78 different culture-density windows during 500 hours. In 2020, Steel and colleagues presented Chi.Bio [38], a platform for automated characterisation and manipulation of biological systems. The research shows that it can be used to study cell growth, biofilm formation, optogenetics and fluorescence emission detection. Chi.Bio is now commercially available.

Interesting extra addons, for more specific biofoundries are also available. Two examples can be found in Box 2.

The power of software and data integration.

Similarly as with lab hardware equipment, computational tools and software resources are present everywhere through the DBTL cycle of a biofoundry as they provide the means for automation and efficient operation. BioCAD (Computer-aided Design) tools are commonly used in biofoundries to explore the metabolic design space, to identify promising pathways and enzymes, and to perform

the design of experiments (DoE) for their combinatorial assembly with DNA regulatory parts, as well as to optimize process conditions [39].

Besides BioCAD tools, lab automation software is essential to bridge models with experimental protocols. This step has long been seen as a bottleneck because most lab equipment generally relies on vendor software that can be hardly integrated into a DBTL pipeline. Such limitations are currently being lifted thanks to the emergence of open-source robotics such as OpenTrons and more awareness from vendors about open biomanufacturing initiatives. Solutions for laboratory digitization such as the open-source Standardization in Lab Automation 2 (SiLA2) [40] used for device communication are also allowing flexible integration of workflows.

Vertical integration of workflows through software and automation interoperability will boost their rapid turn-around. However, one of the bottlenecks in order to automate a biofoundry is the lack of standardisation of software. Most of the vendor software found at biofoundries is proprietary and does not allow for interoperability. Some initiatives like SynBioPython [41] have been recently addressing that issue. Moreover, data integration through software interoperability allows for effective experimental planning based on design of experiments. Such strategy has been advantageously implemented in the manufacturing and chemical industry but is an often-oversight strategy in synthetic biology labs. One of the advantages of data integration is that it allows for the organisation of information and prior knowledge in a way that can be used to optimise the experimental design. Another strategy that should be kept in mind is that rather than focusing on achieving the best titers in the micro well-plates in an automated liquid handling platform, experimental planning should anticipate the challenges of scaling up the process by exploring the design space. Different factors might have different effects depending on the scale. In some cases, exchanging of enzyme variants might lead to important improvements [42], whereas genetic regulation in others can be a key factor in order to increase production [43]. Similarly, changes in process conditions such as media or fermentation parameters might be in some cases critical parameters leading to the most efficient solution. The ability of anticipating which factor would bring to larger improvements at industrial scale is one the major challenges for biofoundries.

Concluding Remarks and Future Perspectives.

The take-home concepts described in this article have been summarised in the Key Figure where we propose an expanded biofoundry lifecycle, hand in hand with the DTBL cycle. Biofoundry facilities have popped up in recent years across the globe with the aim of automating and speeding up synthetic biology in its transition into a biomanufacturing technology for the bioeconomy. Beyond the hype, establishing a successful biofoundry platform requires careful planning studies and should be underpinned by an infrastructure of computational resources as well as DBTL-enabling lab facilities. The human resources may be often forgotten in these plans and are, however, crucial for the success of such an interdisciplinary field. As in traditional IT, training and retaining the invaluable staff is paramount. Personnel can be considered as a more important asset than equipment since well-trained staff provide continuity to projects and funding [5]. Universities play a key role in providing key personnel and, importantly, in facilitating the creation of biofoundries by, for instance, chaperoning the new initiative until funding independence is reached or via incubators or accelerators of entrepreneurial ideas.

Community-wide open-source initiatives including software, hardware and open transfer agreements should help to improve affordability and communication among biofoundries and laboratories as well as to enhance collaborative solutions to meet common challenges (operational, technological and others). Understanding the technological capabilities and limits of those low and mid-end equipment compared with high-cost solutions and addressing other biofoundry-related current issues (see Outstanding Questions), will allow the full exploitation of the true capabilities of these entities. Besides, joint efforts of biofoundries working closely with producers of automation can contribute to the development of new hardware capabilities as it has been already shown in some pioneering experiences [30,31].

Towards smart biomanufacturing, mathematical modelling and computational tools should play a key role to further rationalise, automate, and accelerate the DBTL, identifying those principles that can be generalised as well as enabling a better translation of large-scale conditions to facilitate the scale-up. Importantly, initiatives are currently being undertaken in several biofoundries to address the challenges of scaling and realising economic value at scale. In order to stand out from the crowd, biofoundries should diversify their activities and focus not only on industrial biomanufacturing but also on other cutting-edge engineering biology applications like biocomputation.

Notwithstanding the aforementioned challenges, biofoundries are strengthening their position as hubs of expertise for automation and innovative solutions for the life sciences, as well as for

industrial translation [44], acting also as a bridge between fundamental and applied research. Collaborations with industrial partners (or fees for service work) can actually provide a good sustainability model for biofoundries while helping de-risk industrial delivery. Industry partners may not always be willing to share information, especially what could be advantageous, such as the best technique or method to solve an issue along the pipeline. For instance, the concept of a distributed biofoundry would make more sense—in principle—within academic laboratories. Academic projects are usually driven by public funding and are mainly focused on basic research which define different timescales than industry. However, it is true that current policies defined by major public agencies have brought the spotlight into closing the gap between academia and industry.

While the goals of academic and industrial projects are different, techniques and methods are alike. The use of liquid-handling robots, DNA synthesis or characterisation tools is equally important for both. In a similar way that GenBank was created in the late 70s by an academic laboratory and it is now a basic information tool underpinning many industrial applications, biofoundries could well imply a step-change regardless of the aims of the project at stake. Developing appropriate cost models to make biofoundries widely available is a key to democratising synthetic biology, opening up cutting-edge high throughput experiments to a wider range of groups, including distant research and industrial teams lacking those physical resources in their regions or countries.

Finally, the encouraging role of biofoundries to meet global challenges (health, food, water, energy and environment [45]) should be emphasised. Scientific innovations provide solutions to existing challenges. How are solutions being generated/optimised? What is the balance between new appearing challenges and solution generation? In a time where data analysis, predictions and sophisticated designs are heavily assisted by in-silico tools, the physical generation of new molecular-based solutions is slowed down by trial-and-error procedures. Biofoundries can 1) reduce construction time, and 2) scale-up both complexity and size—from virus vaccines, to crop engineering and bioremediation strategies. Addressing global challenges is an area of activity within the Global Alliance of Biofoundries, which has become particularly relevant during the current pandemic [44], demonstrating how biofoundries are suited to face the shortage of key reagents and could crucially contribute anticipating needs during a global crisis [46].

Outstanding Questions Box

- How can we efficiently train new personnel in the use of automated laboratories and their data integration? How can the valuable staff be retained afterwards?
- How well do DIY and low-cost commercial equipment compare to more consolidated market options? What features (time, efficiency, disponibility of resources…) are being traded off when acquiring each affordable alternative?
- Are all the available computational tools up to the challenge? Are they adapted for an interdisciplinary user?
- Do any ethical concerns arise from the use of biofoundries to solve biotechnological problems?
- Are Universities, academic research centres and the industry ready for this new type of service? What can be done to accelerate the education of possible stakeholders when biofoundries are alien to them?
- How should the biofoundry concept be reshaped in order to be better aligned with current global challenges such as the sustainable bioeconomy?
- What are the challenges for biofoundries in order to become a key player of Industry 4.0 rather than a subsidiary technology, i.e. achieving a biofoundry-based smart circular economy through digitalization, artificial intelligence, IoT, blockchain, etc?

Text Boxes

Box 1. Diversification of biofoundries.

A key outcome of early planning should be the realisation of the possibility to diversify the activities of the future biofoundry. To survive, biofoundries and their defining laboratories must find the niches needed by the community nearby. To do this, the divisions conforming a distributed biofoundry can focus on one main activity and also gain thrust, partners and funding doing other types of biotechnological services that deliver products demanded by the industry if they need to. Now we briefly describe some examples of possible services a biofoundry can provide to supplement its activity.

Amplification of DNA parts and their assembly is a cornerstone of a biofoundry. This kind of cloning service can be demanded by a big range of academic and industrial groups that may need high-throughput cloning, transformation and DNA purification services for any of their projects. The DAMP biofoundry already offers this service in its catalogue (damplab.org).

Protein production is a growing market both for industrial and research purposes where high quality proteins with specific functionalities are needed in big quantities. To find and characterise new proteins, expression and purification protocols must be optimized which often requires a big experimental effort that automated laboratories can help to define. For example, Dudley and coworkers [47] described the expression and characterisation of plant proteins via a biofoundry to improve and speed-up the DBTL cycles towards a more high-throughput protein expression optimization workflow. The system is based on automation to assemble the DNA parts, prepare the plasmids, express the proteins through transformation *in planta* or via a cell-free approach and quantify the expression. Another interesting alternative that would benefit from the biofoundry's services is the microbial-based production of antibodies [48] that can take advantage of combinatorial library assembly to generate antibody variants with new activities [49].

The current volume of prospected and engineered microbial strains and plasmids is pointing at a needed sequencing rate that can only be achieved through the use of automated laboratories that prepare the samples, build the libraries and run the sequencing reactions as fast and precisely as possible. For example, the Agile Biofoundry (agilebiofoundry.org) has incorporated NGS pipelines into some of its laboratories to validate the results of the build phase of their projects.

Finally, the COVID-19 pandemic demonstrated that biofoundries have an important role to play when fast and high-throughput developments are needed to tackle big problems. Biofoundries can help in the diagnosis, as shown by members of the London Biofoundry developed in [50] an alternative diagnostics pipeline and procedure by quickly adapting their automation facility. Additionally, the DAMP Biofoundry developed a large-scale automated testing facility to help in the diagnosis of the virus.

Box 2. Two biofoundry equipment add-ons that can be useful for specific purposes.

3D-printers. For all the steps involved in the normal function of a biofoundry, some project-specific adaptations of the equipment may be required. This can be facilitated by 3-D printers.

The **3D-printing** revolution has reached the synthetic biology laboratories. The possibility to manufacture quick, in-house, custom made, low-cost and complex plastic components for day-to-day life in a molecular biology laboratory is incredibly attractive. Different projects may require very specific elements that can be expensive or may not even exist. Biofoundries face the same problem, exacerbated by the need of high-throughput and the integration between automated components to be as seamless as possible. Some of the previously cited projects use 3D printing to build some of their parts [28–30,35,37]. 3D printers can also be used for more general procedures like the fabrication of custom-made components (e.g. special plates) required for specific pieces of equipment that can be expensive if bought from the manufacturer.

Microfluidics. Applied to synthetic biology, microfluidics has been proposed for various uses in the "build" and "test" phase of the DBTL cycle [51], as a technology to facilitate, automate, miniaturize and reduce the price of fluid manipulation and to reach a fully integrated synthetic biology research pipeline. Much like liquid handlers (but at a micro scale), microfluidics can be used to mix DNA parts automatically and quickly build thousands of constructs. Microfluidics devices however can be expensive to manufacture and operate. For this reason, in 2015 [52] it was presented a 3D-printed microfluidics prototype able to assemble DNA parts through Golden Gate. A low-cost commercial microfluidics kit was presented in 2017 [53] as an affordable DIY circuit building and testing device.

Glossary

- **3D-printing:** 3D-printers use thin threads of melted plastic, added layer by layer to create three dimensional structures.
- **Artificial intelligence:** decision-making strategies through the DBTL cycle based on algorithms with the ability of learning from experimental data.
- **Automated liquid handler:** used to automate the liquid transfer between tubes and plates required to carry out the reactions needed in the engineering process (PCR, cloning, DNA preparation…)
- **Biofoundries:** specialized laboratories that combine software-based design and automated or semi-automated pipelines to build and test genetic devices.

- **Biomanufacturing:** an industrial technology whose main goal is to allow the industries to move from chemical processes to greener, bio-based ones. In its core, biomanufacturing uses biological systems (living organisms, cell extracts, tissues, enzymes) to try to produce valuable molecules

- **Cellular computing / biocomputing:** Cellular computing can be defined as the search for synthetic biological systems that are able to process inputs and deliver outputs according to pre-defined algorithmic rules that are encoded into genetic components.

- **Colony picker:** automated robots designed to identify colonies in an agar plate, pick them and transfer them into other plates for replication or into liquid media for other purposes.

- **Design-Build-Test-Learn:** applied to bioengineering, the DBTL cycle tries to meet a particular design criteria in a biosystem by iterating through the four distinct phases of the cycle applying the obtained knowledge of the previous iterations to better the next designs.

- **Laboratory automation:** set of strategies designed to improve the laboratory methodologies reducing human error, costs and time to production using automated equipment, handing over the baton of tedious laboratory work.

- **Microfluidics:** refers to the study, manufacture and utilization of miniaturized devices interconnected through micro-scale channels designed to manipulate small droplets of fluid from attoliters to nanolitres.

- **Standardization:** developing and implementing synthetic biology standards to maximize modularity, reproducibility, traceability and quality of the engineered systems.

Tables

Strengths Fast response Lowering risks of failure by automation and standardisation	**Weaknesses** Inefficiency of one-size-fits-all solutions Higher uncertainty than its chemical industry counterparts
Opportunities Addressing future demands of the bioeconomy New therapeutics, materials and other valuable bio-based products	**Threats** Quick obsolescence due to a fast-paced technology field High investment in order to reach competitiveness

Table 1. SWOT analysis of establishing a biofoundry. Strong points of biofoundries are that they can provide a fast response thanks to automation and, thus, lower the risk of failure. Weaknesses are mainly seen in the fact that the flexible nature of a biofoundry might render it less efficient than a more focused facility. Similarly, biofoundries rely on biological processes to produce high-value compounds, which are often prone to higher uncertainties in comparison with the production process of the same compound in the chemical industry. Notwithstanding those challenges, biofundries can deliver solutions to address the demands of the bioeconomy.

Political	R&D funding policy. Trade restrictions.
Economic	Economic growth. Exchange, inflation, and interest rates. Investors' interests.
Socio-cultural	Safety perception. Ethical issues. Ageing population.
Technological	Automation. AI. DNA technologies. Lack of specialised workforce.
Legal	GMO regulations. Health regulations. Food regulations. H&S.
Environmental	Climate change. Bioeconomy and circular economy.

Table 2. PESTLE analysis of establishing a biofoundry. The key factors influencing a biofoundry are complex and may arise from the political and economic environments. Moreover socio-cultural, technological, legal and environmental aspects have an impact on the biofoundry and therefore need to be considered in the establishing of a biofoundry.

	Low interest	**High interest**
High power	*Keep satisfied* Funding bodies Finance Consultants	*Manage/Engage closely* Scientific Advisory Board Executive staff Project manager Financial sponsor
Low power	*Monitor* General public Operations/IT Administrative support	*Keep informed* Team members Users Sales Technical sponsor Vendors

Table 3. Stakeholder analysis of a biofoundry. Relevant and interested parties have been identified at the different levels typically found in a biofoundry.

Figures

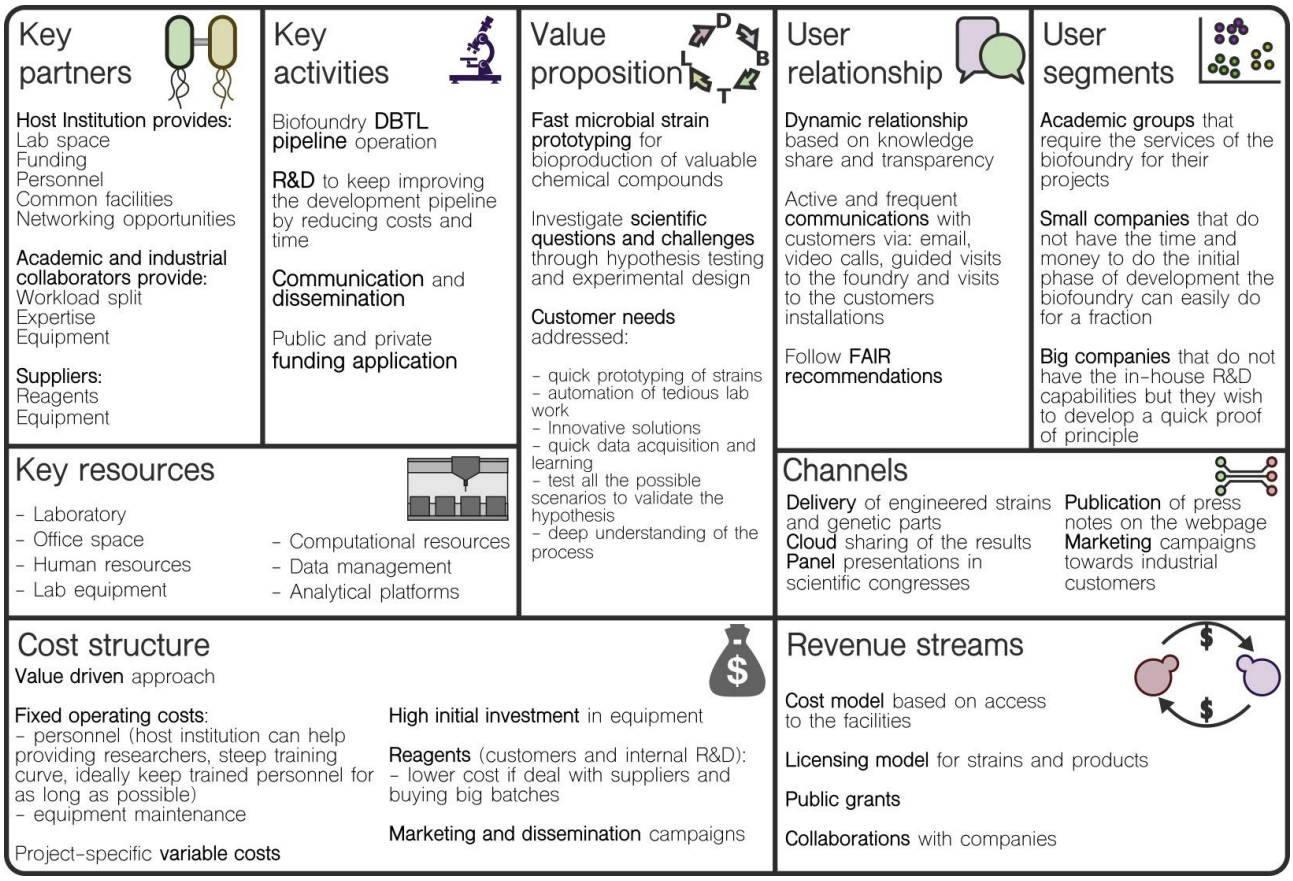
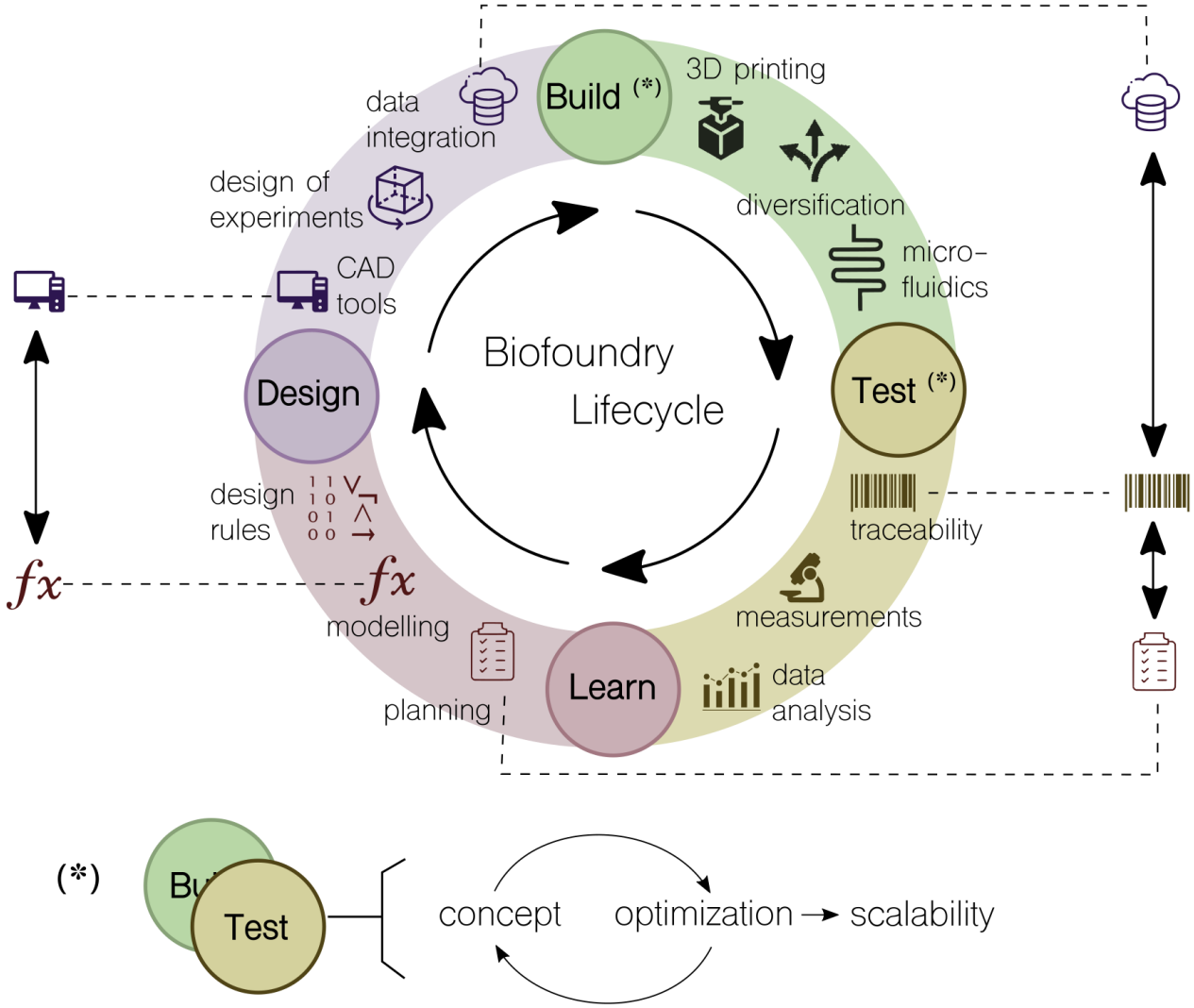


Figure 1. Biofoundry business model canvas. This model has been designed from the perspective of a biofoundry operating within an academic institution. From its inception, the biofoundry needs to define the cooperation with its key partners (e.g. host institution, collaborators and suppliers). Key partners can be of huge help in closing deals, especially during early stages of growth. Another important detail of the canvas is the definition of user of the biofoundry. In essence, a user is anyone interested in the biofoundry's services and that is willing to compensate its work either through direct payment or through the application to funding grants.



425

426 **Key figure.** The complex biofoundry lifecycle. The cycle goes around the DBTL engineering
427 principles, where stage specifics depend on biofoundry capabilities and goals. Two features that go
428 beyond the typical cycle, and are relevant to biofoundry setups, are highlighted here: what we
429 termed extra-cycle interactions (dotted lines), and the loop inside Build-Test that goes from concept
430 to optimization to scalability. The extra-cycle interplay that links modelling (Learn) and CAD tools
431 (Design), for instance, would be the target of a biofoundry that is heavily focussed on
432 characterisation and design. Emphasising the link between data integration (Design) and traceability
433 (Test) would make sense for specific applications, such as evolutionary studies. All these have
434 implications on the type of facilities that are needed. The loop inside Build-Test needs careful
435 considerations in biofoundries that target high volumes of output, such as bioproduction of
436 chemicals.

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