Exploring standards for multicellular mammalian synthetic biology

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Synthetic biology is moving towards bioengineering multicellular mammalian systems that are poised to advance tissue engineering, biomedicine, and the food industry. Despite progress, the field lacks a framework of standards that could greatly accelerate further development. Here, we explore the landscape of standards for multicellular mammalian synthetic biology. We discuss the limits of current technical standards and categorise unaddressed parameters into an abstraction hierarchy. We then define the concept of a ‘synthetic multicellular mammalian system’ and apply our standard hierarchy framework to illustrate how it could aid bioengineering endeavours. We conclude with promising areas that could shape the future of the field, flagging the need for a critical and holistic consideration of standards that requires cross-disciplinary dialogue.

Standards in synthetic biology

Standards are consensus statements on the agreed way to execute a task, communicate information, and quantify a measurement, or formal documents listing guidelines, requirements, and specifications with respect to products and processes. The importance of technical standards in engineering (established during the first industrial revolution) underpins the global supply chain that drives mass manufacturing of goods by predicing product consistency, compatibility, and interoperability, ensuring production efficiency, and minimising defect rates to achieve quality assurance. For example, we can consider an iPhone which is assembled in Taiwan using standard components from across the planet that can communicate with each other reliably and predictably (battery, China; accelerometer, Germany; gyroscope, Switzerland; camera, Japan; LCD screen, South Korea; etc.), then reflect on the contracts in law which stipulate that such subcomponents meet demonstrable standards. As we move into an era of commoditised synthetic biology, it is our view that the field ought to explore what role standards should play in facilitating its successful and responsible development.

Synthetic biology applies an engineering approach to modifying biology, attempting to repurpose or construct de novo biological modules, devices, and circuits for anthropocentric purposes such as biofuel synthesis or biosensing of disease. The accumulation of scientific knowledge and technological advances surrounding genetics and molecular biology has allowed precise editing or construction of DNA, which can be used to introduce custom genes and program functions and behaviours in cells. Efforts have focused predominantly on engineering DNA to program one function in one particular cell type at a time, and as such, standards currently address the technical nuances of DNA manipulation or very specific cell applications [1–3]. However, standardisation has yet to catch up with one of the most exciting and promising facets of synthetic biology: the engineering of multicellular mammalian systems (see Glossary). The programming of complex behaviours that occur in multicellular groups – such as interactions within multicellular consortia, cell-to-cell communication within tissues, or organisation during morphogenesis – has profound implications across science, biomedicine, and food production.

Highlights

Lessons from engineering multicellular systems highlight the importance of considering standards beyond the molecular cloning level.

Synthetic signalling, adhesion, and transcription factor devices have allowed the generation of multicellular entities from a common cell population, or distinct bioengineered populations interacting in coculture. Standards can expedite engineering efforts, output quantification, and stem cell applications.

Synthetic embryology has created embryo-like structures from bioengineered stem cells, advancing our capacity to reconstitute and reconstruct embryogenesis. Standards can improve derivation efficiencies and understanding of these systems.

Cellular agriculture is a rapidly developing concept in the food and feed industries, illustrating the transformative power of synthetic biology and the need for inter-disciplinary standards.

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Unfortunately, conversations are lacking with regard to what constitutes multicellular mammalian synthetic biology and how standards could benefit this sector.

Here, we explore the role of standards in relation to mammalian synthetic biology, concentrating on multicellular entities (e.g., structured tissues or communities) that emerge when functions are programmed into one or multiple bioengineered cell types. The aim of this opinion article is not to prescribe an absolute manifesto for adoption of standards, but rather to explore technical aspects of the broader landscape of standards (Figure 1) and propose directions that are likely to advance the field based on previous successes and current limitations. We hope that this opinion article stimulates readers to consider standards, initiates open discussions, and provides helpful considerations for the engineering of biological systems.

Limitations of current synthetic biology standards

Standardisation in synthetic biology has focused largely on establishing repositories of well-characterised DNA parts (promoters, ribosome binding sites, genes, terminators, etc.) and specialised molecular methods to assemble parts into functional genetic devices with documented efficiencies (protocols) [4]. Plasmid-building systems enable the assembly of standardised DNA parts into mammalian gene expression constructs by exploiting modern molecular cloning technologies, standardising ‘part connectors’, and benchmarking assembly performance [5–8]. These standardised part-and-protocol systems target difficulties related to the custom nature of molecular cloning, but the continuous emergence of such tools implies that none of them serves as a widely adopted standard. This is partially because standardised DNA parts and assembly protocols often cannot accommodate highly specific experimental aims, making custom cloning a necessity.

Importantly, standardised cloning systems cannot guarantee standardised output in mammalian cells. For example, it might seem obvious that maximising transgene expression or inducibility depends on the properties of its promoter [9], and that we can use computational and molecular methods to engineer promoters with desirable properties [10]. Yet transgene expression is also influenced by the relative position of parts across the construct (topology/architecture): when multiple transgenes, each carrying its own promoter, are transcribed in tandem from a common construct, the position of each transgene along the sequence and the activity of neighbouring promoters influence transcriptional outputs [11]. Additionally, different promoters have different propensities to epigenetic regulation [12,13], and their relative performances change unpredictably depending on the genomic integration site [14]. One approach to minimise the locus effect is through standard ‘landing pads’: synthetic DNA elements that, once incorporated into the host’s genome, act as well-insulated harbours for the docking of transgenes [5]. Even for transient expression systems, stoichiometry among multiple transgenes is important in output yield and quality; controlling transcription factor stoichiometry during reprogramming of somatic cells into iPSCs has proved to be critical in optimising reprogramming efficiency and the developmental potency of resultant stem cells [15,16]. These findings highlight the myriad biochemical and biological factors influencing a synthetic device, reflecting the need for a more holistic consideration of standards at the cellular level, rather than standardising the underlying cloning components which could actually restrict manufacturing flexibility and innovation.

How might bioengineers control the plethora of cell parameters that compromise the desired output? If output variation is composed of the variation in all constituent input parameters (e.g., synthesis of artemisinic acid depending on levels of upstream metabolites, availability and kinetics of enzymes of the pathway, etc.) [17,18], then standardisation should initially focus on the few input parameters and rate-limiting factors that account for the majority of output variation.
(the Pareto principle). There are certain challenges to this: context dependency, meaning that what parameter is rate-limiting probably differs across different cell types expressing different sets of genes and possessing distinct physiologies, and orthogonality, meaning that interactions between repurposed genetic components and the reactive environment (the cell ‘chassis’) are unpredictable and controllable only through iterative remodifications [19,20]. While some orthogonality might be achievable through mismatch between transgene and host kingdoms (e.g., expressing plant transgenes in yeast), many mammalian-centred applications require expression of genes in their native context, as maturation of many proteins requires post-translational modifications provided by the mammalian cell apparatus. Hence, standards might be relative to the host cell, which would benefit from the establishment of transcriptomic or proteomic databases characterising the most commonly used mammalian cell lines. This would allow researchers to identify and bypass pitfalls related to host capacity and orthogonality. Alternatively, proactive design of regulative feedback networks could contribute greatly towards reducing variation and improving robustness in genetic devices made from unstandardised parts. Finally, mammalian cells exhibit dynamic instability rooted in epigenetic regulation mechanisms and genetic mutations that drive transgene silencing and continuous culture evolution. Cell-oriented bioengineering solutions to these challenges are actively being pursued, but the current state of cell and tissue engineering calls for a more holistic consideration of standards (Box 1).

To bridge the gap between the limitations of current standards and the complexity of biological systems, we can apply the principles of hierarchical abstraction [21]. We propose an abstraction hierarchy that subdivides the standardisation of synthetic multicellular entities into four levels: genetic, cellular, multicellular, and methodological (Figure 2). The construction and optimisation of anything genetic falls upon DNA engineers, whose outputs are tested in the context of specific cell hosts and genomic loci by cell biologists, who in turn provide characterised bioengineered cells for the construction of multicellular systems. Using this framework as a guiding tool, we can explore how standardisation has benefited multicellular bioengineering so far, and how further progress can be made.

**Synthetic multicellular mammalian systems**

Bioengineering multicellular mammalian systems is a young and promising prospect in synthetic biology [22,23]. We define synthetic multicellular mammalian systems as distinct multicellular entities (e.g., community, tissue, aggregate) that demonstrate an emergent phenotype not observed in the constituent cells individually, and which encompass at least one cell type that has been engineered to possess a genotype pertinent to the systemic phenotype. The multicellular emergent phenotype is commonly patterning, but could theoretically extend to coordinated signalling dynamics or sequential morphogenetic changes that create community-scale behaviours. We categorise synthetic multicellular systems into three general groups: (i) systems that emerge from a single cell population that autonomously exhibits multicellular functions as a result of bioengineering, (ii) systems that emerge from multiple cell types or bioengineered variants that exhibit emergent phenotypes when in coculture, and (iii) technical structures formed as a result of techniques that manipulate physical or chemical properties (e.g., bioprinting, micropatterning, seeding cells onto scaffolds). For the scope of this opinion, we will examine categories (i) and (ii) which deal with genetic–cellular bioengineering where biological standards are most relevant, rather than group (iii) which is rooted in physical and chemical engineering. Biological standards might become important as the field advances and groups begin to overlap, as in the case of bioinspired soft robotics or biohybrid tissue constructs, where embryonic cells and cardiomyocytes amenable to genetic engineering are combined with biomaterial scaffolds to create multicellular systems possessing autonomous or optogenetically controlled aquatic locomotion [24–28].
Single-component systems
Multicellular entities can be constructed by introducing a synthetic genetic circuit that encodes multicellular behaviour(s) into one cell line, creating a single genetic variant that autonomously generates a multicellular community with distinct properties. Examples revolve around signalling systems that bifurcate a common population into separate states [29,30], and transcription factor circuits that mediate cell diversification and segregation into multiple discrete states [31]. These tools could advance tissue engineering by enabling the programming of concurrent fate specifications or the stepwise execution of morphogenetic modules [32].
Box 1. Case studies of standards in cell/tissue engineering

**CAR T cells (chimeric antigen receptor T cells)**

CAR T cells represent the most translationally advanced product of mammalian synthetic biology [84]. In March 2022, the FDA released *Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products*, a draft of recommendations for ensuring product quality, safety, consistency, and compliance with regulatory requirements. For example, section VB recommends that antigen-recognition domains are analysed in terms of affinity, specificity, immunogenicity, and off-target toxicity potential, and that T cells are characterised in terms of cytokine secretion profiling, uncontrolled proliferation, and antigen-dependent cytotoxic activity. These points are critical given that neurotoxicity is the main side effect of CAR T cell therapies. Emphasising the gravitas of standards in safeguarding product critical quality attributes is the case of patient relapse and death due to unintended lentiviral infection of leukaemic B cells with CTL019/Kymriah during CAR T cell manufacturing, which rendered the leukaemic clone resistant to the therapy [88]. Increased attention to standards during the transition from drug to cell therapies is therefore vital. Standards might similarly be piloted for tissue-scale products. Formulating standards proactively can allow scientists to accelerate product characterisation, optimisation, consistency, safety, and regulatory approval. Ultimately, standards can aid in fortifying critical quality attributes and optimising critical process parameters for the manufacturing of synthetic biology products.

**Organoids**

Miniature organs grown from stem cells provide a window into tissue physiology, disease modelling, and personalised medicine. Efforts are now turning to characterising and standardising established organoid models, reflecting the inherent variability which severely compromises manufacturing efficiencies, analyses, and interpretations. For example, autonomous development of self-patterned brain organoids entails a degree of stochasticity that propagates into nonstereotypical anatomy and gross differences in cell composition [86]. Derivation efficiencies vary drastically between batches and cell lines due to, at minimum, nonstandardised culture components, protocol differences, cell line genetics and (potentially) epigenetics [86]. Resulting inconsistencies encumber comparisons between healthy and patient-derived organoids. The emphasis for characterisation is highlighted by the widespread adoption of single-cell transcriptomic analyses to compare organoid composition to foetal counterparts. Standardisation is improving through bioengineered hydrogels, matrices, and culture formats that enable scalable control over organoid size and geometry [87–90]. Unfortunately, cost-effective benchmarking of complex critical quality attributes, such as anatomical order, is limited by the lack of noninvasive standard assays and quantitative metrics. Altogether, the challenges and efforts of organoid research reflect the demand for the reproducible and consistent manufacturing of histologically and anatomically realistic multicellular products, highlighting the critical importance of standards.

In this setting, consistent behaviour of the multicellular entity may directly correlate with standardisation of factors that influence absolute expression, relative stoichiometry, and functionality of transgenes that make the circuit, related to the cell host of choice. For example, in a synthetic lateral inhibition system, cells can adopt a state dominant in ligand presentation through lentiviral vs. transposon vectors. Similarly, for a synthetic Nodal-Lefty2 patterning system and (potentially) epigenetics [86]. Resulting inconsistencies encumber comparisons between healthy and patient-derived organoids. The emphasis for characterisation is highlighted by the widespread adoption of single-cell transcriptomic analyses to compare organoid composition to foetal counterparts. Standardisation is improving through bioengineered hydrogels, matrices, and culture formats that enable scalable control over organoid size and geometry [87–90]. Unfortunately, cost-effective benchmarking of complex critical quality attributes, such as anatomical order, is limited by the lack of noninvasive standard assays and quantitative metrics. Altogether, the challenges and efforts of organoid research reflect the demand for the reproducible and consistent manufacturing of histologically and anatomically realistic multicellular products, highlighting the critical importance of standards.
Figure 2. Abstraction hierarchy for technical standards for multicellular mammalian synthetic biology. The hierarchy entails four levels of ascending complexity (genetic, cellular, multicellular, methodological), and each level similarly entails layers of ascending complexity and operational directionality, from bottom to top. Each level is responsible for the standardisation of its own parameters (layers) in service to the level immediately adjacent, and should be unconcerned for standardisation of parameters belonging to other levels. The multicellular output, such as aggregate morphology or cell composition and patterning, relies on the stoichiometry and total number of cells used to make the system, along with relative growth rates and the bioengineered output of each cell type. In turn, this functional output per cell type (component) is determined by parameters at the cellular level, which utilises DNA constructs produced by the genetic level. The communal aim is the optimisation of the end goal, and dedicated communication channels between levels are needed to facilitate iteration. Abbreviation: GMP, Good Manufacturing Practice.
Multicomponent systems

Composite systems can emerge from distinct bioengineered cell types that interact in coculture. Inspired by the differential adhesion hypothesis, which stipulates that cells differing in adhesiveness can spontaneously sort from one another [34], it was possible to establish self-organising multicellular communities by modulating adhesion on the genetic level [35]. HEK cells bioengineered to synthetically express E-cadherin or P-cadherin sort from each other during coculture, forming phase-separation patterned communities under adherent or suspension conditions [35]. In parallel, the development of a synthetic ligand-receptor system (SynNotch) allowed for the formation of multicellular communities, in which green fluorescent protein (GFP)-presenting ‘sender’ cell clusters instruct surrounding SynNotch ‘receiver’ cells to change their state, forming multicellular communities of radially patterned configurations [36]. Composite devices integrating multiple systems have already emerged, such as cadherin-mediated sorting controlled by SynNotch [37], or the reconfiguration of SynNotch to construct synthetic long-range signalling systems in multicellular groups [38]. These systems allow the programming of multilayered morphogenetic programmes or the rewriting of signalling mechanics, potentially allowing researchers to test principles of embryonic development and advance the current state of tissue engineering [22,39]. In fact, proof of concept that such synthetic systems can be used in conjunction with stem cells to control their differentiation has recently been demonstrated [40,41].

Beyond parameters at the genetic and cellular levels, the phenotype of multicomponent systems also depends on parameters at the multicellular level (Figure 2), such as the relative outputs and ratios between different cell types mixed together. However, it is perhaps metrology that could currently offer most benefit to these systems. In their original work, Foty and Steinberg estimated multicellular adhesiveness by measuring aggregate surface tension using a parallel plate compression method, and correlating the surface tension to the number of cadherins on the cell surface [34]. However, in the synthetic E-cadherin/P-cadherin phase-separation patterning system, it is not known how the two engineered populations differ in surface tension or cadherin numbers, how much difference is required to drive phase separation, or how changes in such parameters affect pattern topography [35]. The work of Foty and Steinberg offers a paradigmatic blueprint for metrology standards: normalise molecular output readings to a standard scale, assay the functional output in traceable units, correlate molecular to functional outputs, and test the model against the multicellular end goal. The incentive for this is that metrology standards entail numerous benefits for experimental design and execution in synthetic biology (Box 2). A significant issue is that multicellular functional outputs, contrary to molecular properties [1,42],

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**Box 2. Benefits of standard metrics**

- Guide rational design and close the design–build–test loop: knowing the amount of functional output needed for a desired result, and how that correlates with the number of molecules per cell, can be used to set a molecule target range and optimise the design to reach the specification.
- Reduce screening time: assaying bioengineered cell lines using standard procedures and metrics accelerates the identification of desired clones, saving time and money.
- Enable quality control: standard assays and metrics are good steps to ensure that synthetic modules and devices are expressed, localise appropriately, and function as intended.
- Improve reproducibility: standard metrics promote standard assays that clarify whether a synthetic behaviour or experiment is reproducible upon repetition or modification.
- Facilitate communication across abstraction hierarchy levels: clear metrics create a reference language that is understandable by all levels of the abstraction hierarchy (see Figure 2 in main text), facilitating communication.
- Accelerate iterations: clear metrics simplify communication towards optimising the desirable output, and allow researchers to clearly understand the effect of iterative modifications.
- Inform theoretical modelling and simulations: using clear, nonarbitrary values from experiments to inform theoretical models builds a better understanding of the process studied, which reciprocally leads to more realistic simulations that expedite design-to-specification and the transfer from *in silico* to *in vitro*. 
are seldom characterised to the extent that standard metrics exist: pattern formation or morphogenesis are emergent properties that seem intuitive to visual perception, with analytic standard metrics being scarce. The development of morphometrics (e.g., structural order in an embryo or tissue) promises notable significance in improving metrology standards for multicellular phenotypes [43], which would facilitate our ability to understand, control, and rationally design multicellular systems to clear specifications. Standard metrics are also informative to theoretical modelling; clearly defined molecular properties have been extensively simulated to understand reaction–diffusion systems and Turing patterns [44], and analogous simulations in adhesion-mediated patterning can guide synthetic morphology [45].

Synthetic embryology: a multicomponent system case study

Synthetic embryology aims to reconstitute and reconstruct facets of embryonic development in vitro by using stem cells to build embryo-like model structures (stemembryos) [46,47]. While some models emerge through technical manipulations (e.g., timed provision of ligands or micropatterning) [48–51], some of the most recent systems comprise multiple stem-cell types brought together in coculture, with synthetic overexpression of transcription factors being used to advance them further [52–54]. These latter entities are multicomponent synthetic multicellular systems. The wealth of different models has sparked discussions about the need for a commonly accepted naming scheme [55], and to delineate how each model relates to true embryogenesis through a notional developmental landscape [47]. This is, sensu proprio, a call for standards: the naming convention is a metadata standard, the embryo is the reference standard, and the developmental landscape would be a standard map. These standards could delineate the relevance and importance of these models, and assist scientists in understanding fundamental principles of embryogenesis.

The map and its scale are likely to be based on transcriptomic comparisons at various developmental points, as exemplified by studies comparing temporal gene expression between stemembryos and natural embryos [54,56]. This is being pursued in organoid research, where organoid single-cell RNAseq data are being mapped to reference databases or in situ hybridisation atlases of in vivo counterparts, which allows estimation of developmental stage, annotation of regional identity, deconvolution, reconstruction of differentiation trajectories, and exploration of unknown cell populations in organoids [57–62]. The launch of the Organoid Cell Atlas for cell characterisation and data research in human organoids exemplifies the utilisation and establishment of transcriptomic databases as reference standards [63]. Indeed, the bioinformatic comparison of organoid datasets to organ transcriptomic databases, followed by the application of synthetic biology to correct discrepancies, is beginning to be used to improve organoid maturity [64]. It is not inconceivable that a similar framework will apply to synthetic embryology, given that a single-cell transcriptomic map of gastrulation is available [65].

On a technical level, creation of desirable stemembryos is inefficient as stem-cell-derived aggregates are heterogeneous, which might be ameliorated by considering multicellular standards. For example, iETX stemembryos recapitulate critical events leading to the formation of the mammalian body plan, providing an unprecedented opportunity to investigate principles of embryogenesis and create embryos in vitro. The pursuit of this opportunity is hindered by the fact that a small fraction of multistem-cell aggregates develop into iETX stemembryos [34]. This raises the question of whether all aggregates are made the same, with respect to total cell numbers, cell stoichiometry, and number of cells synthetically expressing low or high transcription factor levels in an aggregate, which depends on the distribution of inducible output levels and thus bioengineering standards (Figure 3). These considerations reflect standards at the ‘multicellular’ and ‘cellular’ levels of the abstraction hierarchy (Figure 2). Endeavours to standardise bioengineered cells and aggregates
Figure 3. Multicellular synthetic biology standards for multicomponent systems, using iETX embryos as an example [54]. Multicellular output: three components (trophoblast stem cells, embryonic stem cells, GATA4-inducible embryonic stem cells) are used to make 3D multicellular aggregates, of which a small percentage yield synthetic iETX embryos. Output-per-component: if the transgenic line shows cell-to-cell variability in GATA4 induction levels, and assuming that conversion to primitive endoderm requires cells to express GATA4 above a certain threshold, then different aggregates carrying the same numbers of GATA4-inducible cells can have different proportions of primitive endoderm by means of sampling error. Stoichiometry of cells: different aggregates can have variable composition in terms of tropoblast:embryonic:GATA4-embryonic stem cell ratio (1, similar representation per component; 2, under-representation of GATA4-inducible embryonic stem cells; 3, under-representation of trophoblast stem cells). Growth: differences in growth rates among component cells could theoretically lead to different multicellular outcomes (1, outcome if trophoblast stem cell growth rate outcompetes other components; 2, outcome if embryonic stem cell growth rate outcompetes other components; 3, outcome if GATA4-overexpressing cell growth rate outcompetes other components). Total number of cells: aggregate capacity to yield the desired iETX phenotype might require aggregates to fall within a specific range of possible sizes, which is directly related to the total number of cells initially combined to form an aggregate.
will be important to boost efficiency and understand the nuances of these stem-bryos, similar to the importance of standardised culture components, serum, growth factors, and extracellular matrix substrates in safeguarding reproducibility. Stem-bryos and transplantable organoids are still far from the general consumer; framing standards for commercialisation of synthetic multicellular products requires a near-market example.

Standards for cellular agriculture

The concept of ‘cultured meat’ – cultivating animal biomass intended for consumption using engineered cells grown in bioreactors – is rising in popularity due to concerns around animal welfare, the environmental impact of meat production, and food security for an increasing human population [66,67]. Technically, cellular agriculture seeks to derive high yields of animal skeletal muscle cells from various sources such as primary muscle stem cells, embryonic stem cells, or transdifferentiated somatic cells. Occasionally, marine animal cells and fungal mycelia are also promoted as ‘cultured meat’. With the exception of fungal mycelia, cultures can be grown up to a few millimetres thick using various scaffolds [67–69]. Cultured meat is unlikely to provide the same mouthfeel and nutritional value as natural meat in the near term [66,69]; attempts to incorporate adipocytes into the process have only just begun [70]. Along with product composition, it is also unclear what metrics are being used to benchmark myotube derivation and quality assurance (e.g., percentage of multinucleate cells, α-actinin-based myogenic index), or how chemical agents present during manufacturing (e.g., hormones, antibiotics) carry over to the final product. Since animal ethics and sustainability are two of the industry’s main drivers, companies should report expenditures on energy, water, and carbon emissions per kilogram of biomass produced synthetically relative to that produced naturally, while achieving large-scale serum-free manufacturing [69,71]. Overall, standards are needed to address product composition, quality, safety, and manufacturing practices (Figure 4). However, it might be prudent not to rely entirely on standardised cells, as the emergence of a drug-resistant parasite adapted to a master cell line could readily spread among genetically identical cultures [20].

Legislation and regulation standards for cellular agriculture are under development. In the USA, the FDA and the US Department of Agriculture/Food Safety and Inspection Service (USDA–FSIS) will jointly regulate virtually all manufacturing materials and processes, including packaging and labelling. The specifics are unclear, but cellular agriculture practitioners can study guidelines for the qualification of cell substrates used in the production of monoclonal antibodies or viral vaccines. These standards require that master cell banks, working cell banks, and end-of-production cells are tested for culture sterility with respect to bacteria, fungi, viruses, and clearance from viral vectors and endotoxins. Species-specific tests might be included, such as sterility of cultured chicken cells from salmonella or Rous sarcoma virus. Cell line authenticity – including species of origin, identity, and lack of cross-contamination – is also requested, and this might become crucial to certify protected designation of origin products.

It remains unknown whether guidelines for intentional alteration of genomic DNA and integration of recombinant DNA in animals will apply to cellular agriculture. According to the FDA’s guidance for industry (#187 draft), DNA articles ‘intended to affect the structure or function of the body of the animal’ qualify as new animal drugs. To be deemed safe, each drug requires a New Animal Drug Application (NADA) approval. Each specific DNA alteration at each specific locus qualifies as a new drug and requires a separate NADA. If this transfers to cultured meat, separate transformation events or populations harbouring DNA alterations in different loci will require separate NADA approvals. Furthermore, genetically engineered animals are subject to phenotypic sampling over noncontiguous generations, and compositions of edible tissues must be shown to be as safe as that of commonly and safely consumed counterparts. If this applies to cultured meat, consistent
## Starting material

| Kingdom of input cells | Starting cell type | Working cells |
|------------------------|--------------------|---------------|
| Terrestrial animal     | Adult stem cells   | Line: .......... |
|                       | Somatic cells      | Passage number: .......... |
|                       | Embryonic stem cells | Ploidy: .......... |
| Marine animal          | Induced pluripotent stem cells | Provider: .......... |
| Fungal                 | Other: .......... | Species of origin: .......... |

### Method

| Genetic modification | Chemicals used | mg per 100g product |
|----------------------|----------------|---------------------|
| Genomic alteration   | Antibiotics: .......... | .......... |
| Genomic alteration   | Hormones: .......... | .......... |
| Genomic alteration   | Additives: .......... | .......... |
| Immortalization      |                |                     |

### Product

| Cell types in product | % of final product | Screening assay/metric |
|-----------------------|--------------------|------------------------|
|                       |                    |                        |

| Costs per kg product | Nutritional content | Per 100g product |
|----------------------|---------------------|-----------------|
|                      | Energy              |                |
|                      | Fat                 |                |
|                      | Carbohydrates       |                |
|                      | of which sugars     |                |
|                      | Fibre               |                |
|                      | Protein             |                |
|                      | Salt                |                |

### Regulatory

- Compliance with good manufacturing practices
- Culture sterility certification

_Trends in Biotechnology (See figure legend at the bottom of the next page.)_
cell line behaviour and product composition will be integral to product approval. Industry-led standards could address site-specific genomic alterations, genetic and phenotypic characterisation of clonally expanded lines, robust behaviour of bioengineered lines, and consistent product composition. For the labelling of genetically engineered products, legislation standards include the US congress report R46813 covering the National Bioengineered Food Disclosure Standard (Public Law 114-216; 2016 Act), and the EU Article 8(1)(a) of Novel Foods Regulation [72,73]. Labelling standards are crucial for cultured meat as public outlook is strongly polarised, with early adopters placing high value on government seals of approval [74–76]. Naming schemes can invoke positive behavioural intentions without clarifying the product’s origin (e.g., ‘clean meat’ vs. ‘lab-grown meat’), suggesting that labelling standards must protect nonadopters from misleading marketing [77]. The AquAdvantage Salmon, which achieves rapid growth due to a stably integrated transgene driving expression of a Chinook salmon-derived growth hormone, represents a paradigm for the regulation of genetically engineered animals.

Concluding remarks
Advances in synthetic biology will lead to unprecedented scientific breakthroughs and novel consumer products (e.g., synthetic embryos, improved organoids, ‘cultured meat’). For scientists and bioengineers, pragmatic consideration of technical standards can be guided using an abstraction hierarchy (Figure 2). Establishment of cell transcriptomic databases (reference standards) could assist scientists to bypass context dependency and orthogonality pitfalls, while metrology standards could facilitate rational design, improve control over molecular and phenotypic outputs, increase bioengineering success rates, and clarify communication among scientists and between industry and the public. However, we argue that standards should be evaluated critically and not slavishly adopted; reaching a standard performance score (benchmark) and safety assurance through unrestricted flexibility might be counteracted by standards that are misused to limit creative experimentation and the availability of manufacturing components. The aforementioned points might be relevant not only to tissue-scale systems but also to the bioengineering of eukaryotic cell subcompartments (e.g., organelles) or whole multicellular organisms (e.g., genetically modified mosquitoes) [78,79].

To close, we highlight that the successful and responsible development of this new bioengineering wave will rely on an interdisciplinary landscape of standards, calling experts across diverse fields to come together and shape its future (Figure 1). Steps to address different facets of this scope are being made [80–83], and we encourage further cross-disciplinary discussions given the potential of synthetic biology to create disruptive tools that will radically advance our control over major biological and biomedical processes. We hope to motivate experts across the bioengineering community to come together at devoted symposia, identify strategic solutions to major limitations, and establish collaborative research teams aimed at improving bioengineering standards through dedicated funding (see Outstanding questions).

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Outstanding questions
Can mammalian cell hosts be characterised and standardised through collaborative projects, dedicated funding, and nonprofit cell repositories? Transcriptomic or proteomic databases could be consulted as standard references to assess host capacity and orthogonality for successful bioengineering efforts. For multicellular systems, atlases of embryos and organs similarly act as standards to evaluate the realism of synthetic embryo models and organoids.

Can standard components or computational logic networks standardise the behaviour of synthetic devices to robustly activate gene regulatory networks and signalling circuits? Standards to optimise such devices will be integral to ensuring robust performance and to push them towards the clinic or market.

What standard metrics can be formulated to quantitatively describe complex multicellular phenotypes (e.g., anatomical order)? This calls for international collaboration among biologists, engineers, and (bio)physicists drawn from academia, industry, and national measurement institutes. Metrology advances could extend beyond synthetic biology to benefit other fields, such as high-content organoid screening.

How should legislation and regulatory standards prepare the road for potential synthetic biology products and applications reaching the public? Legislation and regulatory bodies might enact standards extending across ethical cell sourcing, cell line certification, serum-free manufacturing, sustainability, product labelling, and documentation of nonproprietary information.
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