NIST Special Publication 1500-13

CELL-FREE (Comparable Engineered Living Lysates for Research Education and Entrepreneurship) Workshop Report

Eugenia Romantseva
Elizabeth A. Strychalski

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.1500-13

National Institute of Standards and Technology
U.S. Department of Commerce

NIST Special Publication 1500-13

CELL-FREE (Comparable Engineered Living Lysates for Research Education and Entrepreneurship) Workshop Report

Eugenia Romantseva
Elizabeth A. Strychalski
Cellular Engineering Group
Material Measurement Laboratory

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.1500-13

February 2020

U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Walter Copan, NIST Director and Undersecretary of Commerce for Standards and Technology

National Institute of Standards and Technology Special Publication 1500-13
Natl. Inst. Stand. Technol. Spec. Publ. 1500-13, 25 pages (February 2020)
CODEN: NSPUE2

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.1500-13

Abstract

The CELL-FREE Workshop sought to identify and prioritize actionable steps towards more reproducible and comparable cell-free systems for practical applications in bioengineering and biomanufacturing.

Findings

- Cell-free systems generate broad excitement for their potential as an enabling technology platform, but their full capabilities and suitable applications remain unclear.
- A common repertoire of protocols and methods for typical cell-free systems will aid adoption and reproducibility.
- Improved access to data and sharing of information and expertise across laboratories will also aid adoption and reproducibility.
- Improved methods and tools for measuring the components and performance of cell-free systems at all stages in a typical workflow from reagent preparation to final product are needed to advance reproducibility and applications of cell-free systems.
- Cell-free systems may differ from cell-based systems in ways that significantly impact the performance of cell-free systems, when protocols, materials, and measurements for cell-based systems are applied naively to cell-free systems.

Recommendations

- Focus the research community and spur investment by identifying specific foundational studies and application areas well-served by cell-free systems.
- Develop and disseminate standard protocols for DNA template and lysate preparation for common use cases.
- Encourage engagement with existing online resources for community and information sharing, such as the BuildACell/CellFree page on OpenWetWare [1].
- Perform interlaboratory studies to identify and test best practices for reproducible lysate and DNA template preparation.
- Develop a standard test plate and protocol to assess reagent quality and facilitate characterization, performance, and reproducibility of cell-free reactions.
- Rigorously assess all assumptions associated with the use of protocols, materials, and measurements for cell-based systems applied to cell-free systems.

Key words

Cell-free systems; TX-TL; in vitro; engineering biology; synthetic biology

Table of Contents

1. **Motivation** 1
2. **Approach and Organization** 2
3. **Modeling Working Group** 2
4. **Lysate Preparation Working Group** 4
5. **DNA Template Preparation Working Group** 5
6. **Measurement Needs Working Group** 7
7. **Sharing and Reporting Information Working Group** 12
8. **Findings** 12
9. **Recommendations** 13
10. **Outlook** 13

References 13

Appendix A: Supplemental Materials 17

List of Tables

Table 2. Workshop Agenda. 17
Table 3. Workshop Participants. 18

List of Figures

Fig. 1. Towards reproducible cell-free systems. 1

1. Motivation

> ***"In principle, cell-free systems are simpler [than cell-based systems], but in practice, that hasn't been true, at least not yet."*** – Matthew Lux, workshop participant and researcher using cell-free systems [2]

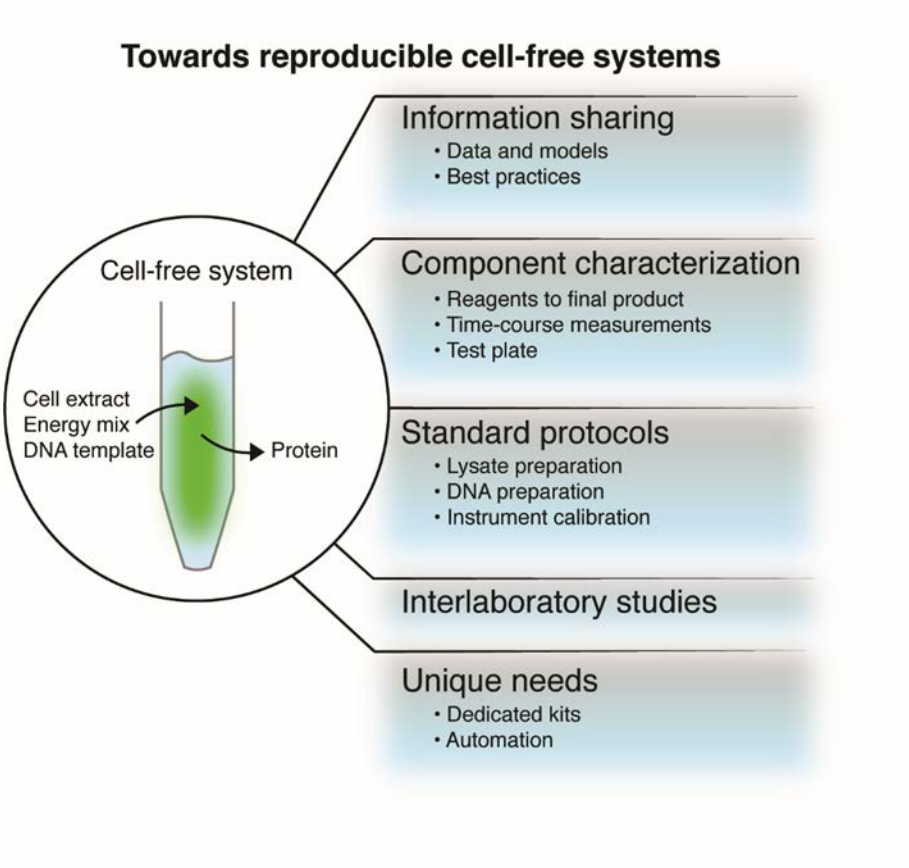

Fig. 1. Towards reproducible cell-free systems.

Living systems are not only complex, they are relentless in their demands to be kept alive, with all the attendant limitations and challenges for experimentation and measurement. So-called "cell-free systems" inhabit the space between chemistry and biology proper, obviating some of the requirements of living, cell-based systems and enabling biochemical processes and experimental interventions toxic, contrary, or simply inconvenient to the enterprise of being fully alive. Cell-free systems are typically composed from the minimally-prepared extracts of lysed cells or reconstituted from purified biochemical components, supplemented with the desired DNA template and a chemical mixture to supply energy, amino acids, and small molecules to the system. A recent review of current organisms, methods, and applications is offered by [3].

Despite a relaxation of the stringent demands of living, users of cell-free systems must still contend with the inherent complexity of life that is both the compelling strength and confounding liability of cell-free systems. Consequently, a lack of reproducibility persists within and across laboratories engaged in research using cell-free systems. Complexity in

cell-free systems can manifest as variability in function, such as protein production, which generally cannot be designed out or optimized away at the state of the art. Although biological variability undoubtedly contributes to generally poor reproducibility in uses of cell-free systems, this Workshop focused instead on modeling, laboratory practices, and measurement technology to improve reproducibility. Biological variability may then be approached as an enabling feature of engineered biological systems for emerging applications. This view deliberately tempers a pervasive myopic affinity for deterministic systems, which would miss the opportunity to learn control approaches unique to biological systems and harness these for safe and robust applications of engineering and synthetic biology broadly.

Stakeholders of the National Institute of Standards and Technology (NIST) in industry, government, and academia have requested measurement tools and methods to improve reproducibility, primarily for protein expression using cell-free systems, to support intra- and interlaboratory collaboration, assist nascent industry, and improve fundamental understanding of biology. As part of a broader portfolio of investments in synthetic biology and engineering biology, NIST is building technical competence and measurement capabilities in cell-free systems. This Workshop and report constitute early outputs of these efforts.

2. Approach and Organization

The need for this Workshop became clear in conversation with numerous stakeholders across the community of cell-free researchers. Following the example of existing online discussion forums initiated by, for example, the Murray laboratory [1, 4] and Build-a-Cell [5], NIST hosted documents online to gather relevant information [6] ahead of the in-person meeting. This online interaction enabled the open exchange of ideas, concerns, and so on, regarding the formation of Working Groups and the Workshop agenda, as well as facilitated the exchange of notes and other materials after the Workshop. Working Group topics were selected for their immediate relevance to the central task of identifying actionable, near-term steps to improve reproducibility for cell-free systems. Working Group leaders were chosen for their knowledge and respected standing in each of the Working Group topics. A summary follows, collated from notes taken during each Working Group discussion. This Report offers the opinions presented by participants, as captured through this imperfect process. The authors of this Report make no claims to a comprehensive or balanced survey of the field of cell-free systems broadly.

3. **Modeling Working Group,** led by William Poole (California Institute of Technology) and Richard Murray (California Institute of Technology)

Several general goals and guidelines surfaced during discussion in the Modeling Working Group. First, modeling efforts should aim towards predictive, rather than simply descriptive, models of cell-free systems. Second, models should build understanding and inform capabilities that bridge in vitro, cell-free systems and in vivo, cell-based systems. Third, models should be compatible with practical experimental testing according to existing capabilities, to ensure relevance and speed model validation. Fourth, specific, near-term applications of modeling include aiding in the design of biological circuits, optimizing resource sharing and substrate blocking, improving protein folding, and realistic models of

metabolism. Last, with regard to reproducibility, modeling should inform both fundamental understanding of biology and practical approaches to tune variability in the performance of cell-free systems for protein production.

A variety of biological systems were discussed as potential focal points for modeling efforts for cell-free systems, along with the advantages and challenges of each system. Today, efforts produce largely generic models applicable to extracts from common laboratory organisms, such as *E. coli* and the reconstituted PURExpress system[1] [7-12]. While cell extract includes biological complexity ignored and absent from models, the PURExpress system itself arguably avoids much of that complexity altogether. The PURExpress system may therefore serve as an attractive and tractable intermediate step towards modeling more complex lysates, composed of cell extract supplemented with an energy buffer, or chemical mixture to supply energy, amino acids, and small molecules to the system. The behavior of DNA, RNA, and metabolites could be modeled in the PURExpress system, but not all relevant mechanisms and parameters are currently well-defined.

To move from an understanding of the PURExpress system to extract-based and in vivo systems, participants recommended incorporating explicitly the effects of molecular crowding. Because molecular crowding changes biomolecular dynamics within a biological system, this potentially offers a practical experimental approach for testing and optimization. A well-mixed model may not adequately account for affects due to molecular crowding. Rather, spatial structure should be modeled at spatial scales associated with relevant interactions within the cell-free system. However, the spatial structure of cell-free systems at different length scales and over time remains unclear, and control over this structure, for example through molecule scaffolds or microfabricated environments, could provide another experimental tool for optimization.

Participants recommended that models of cell-free systems also include time dependence. Models could predict the performance of a cell-free system throughout the life cycle of the reaction, and experimental data at regular time points could be obtained in a straightforward manner. Today, most cell-free systems are measured only after the reaction has run to completion, but more frequent measurements, especially for early time points, could provide important data to inform time-dependent, predictive models. Such models could address resource utilization, inform methods to remove detrimental byproducts, troubleshoot how reactions “die,” and offer paths towards increasing or otherwise tuning reaction longevity.

This Working Group called for the generation of new experimental data and sharing data from existing studies to inform more accurate, useful, and predictive models. Standardization of experimental samples and protocols could assist in comparability across datasets and model development. Integrated data management tools and artificial intelligence could help to collect, store, and analyze the simulated and experimental data. Ideally, experimental data and models would be accessible broadly in a repository. However, using, integrating, and modifying existing models remains challenging, with no standardized language, code, or methodology in use. Existing options include, for example, subsbml to combine models [13],

[1] Certain commercial equipment, instruments, or materials are identified to adequately specify experimental procedures. Such identification implies neither recommendation nor endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

SED-ML for sharing simulations [14], the COMBINE archive for documenting models [15], and SBML for sharing models [16].

4. Lysate Preparation Working Group, led by Zachary Sun (Tierra Biosciences [17])

This Working Group gave a bleak characterization of the state of lysate preparation for cell-free systems, which is largely non-standardized, expensive, and irreproducible. Laboratories widely choose to produce their own lysate, even for common organisms, because existing commercial kits are prohibitively expensive, have limited applications, and are not customizable. Commercial kits do not seem to be any more reproducible or characterized than their laboratory-prepared analogs. Consequently, each laboratory typically has one person with so-called "magic hands" who, for undetermined and perhaps indeterminable reasons, is best able to produce suitable lysate for all users and collaborators for that laboratory. Protocols tend to follow historical precedent without optimization, suggesting the possibility that the full range of performance attainable by cell-free systems through optimization of the lysate remains unexplored and potentially considerable.

It is unclear the extent to which reproducibility in lysate preparation may contribute significantly – if at all – to reproducibility in the ultimate performance of a cell-free reaction. Complicating the matter is that no accepted criteria exist to determine how reproducible is reproducible enough generally or for a given application or purpose. Lysates are inherently heterogeneous, variation between nominally identical batches is common, and even nominally identical lysate from a common batch stored in different aliquots may perform differently. Equipment for preparing lysate varies across laboratories, such as sonication, bead beating, French press, and others, and operation of the same type of equipment varies between laboratories. In one study, sonication was more reproducible than other methods [18, 19]. Beyond hardware, human operators are reportedly a large source of variability [CCDC Chemical and Biological Center manuscript in preparation], the length of typical protocols offer many opportunities for operator error, and the level of detail in typical protocols leaves room for interpretation.

Consider, for example, the numerous factors that may introduce variability in uses of *E. coli* lysates for cell-free systems. The choice of *E. coli* strain and whether a runoff reaction [20] was performed generally affect the final cell-free reaction. Cell growth is affected by media composition, and the use of defined media could help standardize the preparation of cell lysates. Researchers may find, for example, that B strains of *E. coli* tend to perform better than K strains for no discernible reason. Similarly, researchers may prefer lysate derived from cells grown at nominally different incubation temperatures. Lysates may be further affected by the specific starter culture, presence of phage, use of a glycerol stock, use of an ill-defined "overnight culture," technique for colony picking, pellet lyophilization, amount and composition of gas headspace in culture vessels, type of flasks, shaking speed, optical density at harvest, lysis method, and use of clarification steps. Generally, cells harvested from exponential growth through stationary phase have been shown to work adequately for lysates as part of cell-free reactions [21]. Measurements of optical density should be calibrated properly to ensure reproducibility for cell growth prior to lysis [22].

Participants in this Working Group called for interlaboratory studies to assess lysate composition, performance, and reproducibility to improve the state of the art. Similar work

with reference yeast strains [23], oligonucleotide microarrays [24], and a triservice interlaboratory study [25] could serve as guiding examples. The study would perhaps compare the performance of an *E. coli* cell lysate prepared using a common protocol across interested laboratories. Several detailed protocols are available, such as the Murray protocol in JOVE [26], which was the first known attempt to standardize a protocol with an associated video that demonstrates each step, the Hasty protocol [27], and protocols from the Jewett laboratory [18, 28]. Potential parameters to standardize in a protocol for lysate preparation could include, for example, protein abundance and activity, concentration and purity of supplemented energy, amino acids, small molecules, amount of residual native genomic DNA, and the use of crowding agents. This is envisioned as a collaborative endeavor partnering academic researchers, government agencies, and private industry.

In addition to improving lysate preparation within individual laboratories, this Working Group advocated for a shared production facility for cell lysate to advance accessibility, affordability, and reproducibility of cell-free systems. For example, a non-profit cost center may produce standardized and characterized lysate to meet the needs of the majority of users working with cell-free systems. The center could encourage publications in optimization approaches that improve reproducibility and performance. Lysate produced from *E. coli* is an obvious initial focus, along with promoting good practices and protocols to modify standardized lysates for specific application areas of common interest. In this way, the cost to produce cell lysate of sufficient quantity, quality, and characterization suitable for most cell-free applications may be reduced. Currently, the main cost to produce cell lysate is labor, which presumably inflates the cost of commercial kits from an ideal cost of approximately \$0.03/μL [26] to, for example, prohibitive values currently of \$0.88/μL for myTXTL[2] [29] and for \$1.044/μL for the PURExpress system [7].

5. **DNA Template Preparation Working Group,** led by Vincent Noireaux (University of Minnesota)

Active discussion surrounds the topic of DNA template preparation, despite the wide availability of commercial kits and extensive knowledge and experience accumulated in the biosciences for this purpose. Perhaps commercial kits for DNA preparation for cell-based systems would benefit from modification and optimization, such as improved quality control, additional clean-up steps, and product pamphlets with instructions specifically aimed at researchers using the prepared DNA in cell-free reactions, to yield DNA templates better suited for use with cell-free systems. Participants in this Working Group asserted that accessible, standardized DNA preparation protocols would facilitate the wider adoption of cell-free systems. Developing such protocols would require guidance and methods for relevant quantitative measurements. Variability in the performance of cell-free systems could arise, in principle, not only from the amount of DNA template introduced into a cell-free reaction but also from the quality of the DNA itself and contaminants in the solution containing the DNA.

Improvements to quantifying DNA templates are straightforward with existing measurement technologies and good laboratory practices. For example, rather than assume that prepared

[2] Certain commercial equipment, instruments, or materials are identified to adequately specify experimental procedures. Such identification implies neither recommendation nor endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

DNA does not change during storage, the authors of this Report observed an approximate 10 % mean change in the concentration of extracted and purified DNA using high copy number, 3.2 kbp plasmids when stored for 30 days at 4 °C in nuclease-free water in LoBind tubes[2], as measured using both the Nanodrop[2] and the Qubit Broad Range Assay[2] (unpublished). Consultation with experts in DNA storage at NIST [30] revealed that this loss of DNA should have been expected, due to adsorption of DNA to the tube surfaces and/or hydrolysis of the DNA in water. Teflon tubes and storage in a low concentration salt buffer could mitigate this loss [30] but may not be realistic, due to the prohibitive cost of the custom tubes and potential effects of the buffer salt in the subsequent cell-free reaction. Mitigation may not even be necessary once the effects of DNA loss are acknowledged, quantified, and compensated for when assembling cell-free reactions. In this case, the prepared DNA was likely still adequate for most cell-based purposes, but the change in DNA concentration may have impacted the measured amount of protein produced when added to cell-free reactions (unpublished). Measurements of DNA quantity during this process were obtained using ultraviolet spectrophotometry (Nanodrop [31]) and fluorometry (Qubit Assay [32]) [33] calibrated using the NIST Human DNA Quantitation Standard [34].

Beyond the quantity of the prepared DNA template, the quality of each DNA molecule may impact whether that molecule is functional in a cell-free reaction. Typical measurement methods for the amount of DNA may not accurately represent the functional amount of DNA, whether impacted by physical damage, chemical modification, or other changes to a DNA molecule. Both plasmids and linear DNA templates, especially for DNA molecules longer than approximately (103 to 104) bp, are susceptible to shearing and breakage from pipetting, vortexing, passage through separation columns included in some common kits for DNA template preparation, and other manipulation. Some researchers with experience with cell-based systems may disagree, perhaps due to typical cell-culture and colony selection steps that select out broken or otherwise nonfunctional DNA. Still, most researchers agree on the difficulty in handling very long, genomic DNA, which is possible but imposes practical limits at the state of the art on delivery and use of that DNA for applications in cell-free systems. Genomic DNA may be delivered, for example, encased in agar or another gel matrix, but the additional processing steps add unwelcome complexity to experimental protocols [35].

The aqueous solution in which the DNA template is suspended during preparation and assembly in a cell-free reaction may also influence the functional amount of DNA. Consider that a significant volume of a typical cell-free system is the DNA solution. For example, a 12 µL myTXTL reaction may require 5 nM DNA, which, for the authors, was approximately 1 µL DNA solution comprising a considerable 8.3% of the overall volume of the cell-free reaction (unpublished). Contaminants are known to pass through steps of the DNA purification and preparation protocols and remain in the final solution containing the DNA template as added to the cell-free reaction. At nearly 10% of the final reaction volume, this may be a significant concern in cell-free workflows that accumulate contaminants and consequences from all preparatory steps for each ingredient in the final reaction. Because plasmid DNA templates are purified from cells, DNA plasmids are arguably more susceptible than linear DNA templates to contaminants that may not be adequately removed during extraction, such as salts and Ribonuclease A (RNase A).

Anecdotally, plasmid DNA extracted from cells using various commercial kits give different and inconsistent results when used in cell-free systems. Workshop participants report that midi and maxi prep kits [36-38][3], although more time consuming than mini prep kits [38, 39][3], generally yield DNA templates that perform better for protein production using cell-free systems than mini prep kits, which require additional purification steps for DNA templates of suitable quality. The Murray laboratory [4] improves the quality of DNA templates after extraction through three consecutive ethanol washes, while the Noireaux laboratory [40] purifies DNA templates after extraction with a cleanup kit developed for use with polymerase chain reaction (PCR) products to obtain a higher protein yield relative to that same DNA template prepared without the additional purification step [41]. Unfortunately, these approaches increase the length of protocols for DNA template preparation, allowing correspondingly more opportunities for DNA breakage, DNA loss, and operator error.

Currently, DNA templates for cell-free systems [42] are typically plasmid DNA, despite the increased labor and time required for preparation as compared to linear DNA. Working Group participants noted that protocols that enable the routine use of linear DNA templates in cell-free systems are highly desirable. Linear DNA is susceptible to degradation in most lysates due to the RecBCD complex, an exonuclease in *E. coli* extracts essential for double-strand break repair [43]. Two successful approaches to protect linear DNA in cell-free systems from degradation by endogenous recBCD use the bacteriophage protein GamS [44] and modified linear dsDNA with six χ-sites (Chi6) [45]. Unproven, impractical, and/or unoptimized alternatives to these include using a modified *E. coli* that removes RecBCD but leads to decreased lysate performance, the addition of small molecules to inhibit RecBCD, and unpublished work suggesting linear DNA modified with unnatural bases may prevent degradation.

6. **Measurement Needs Working Group,** led by Eugenia “Jane” Romantseva (National Institute of Standards and Technology)

The Measurement Needs Working Group focused on determining the specific measurement tools, methods, and needs for more reproducible outcomes in cell-free systems. The topics pertained to many aspects of cell-free systems and overlapped substantially with discussions in the other Working Groups. The summary provided here would benefit from a more comprehensive treatment to arrive at prioritized recommendations to best guide the field. The Measurement Needs Working Group identified four areas of the typical workflow for cell-free systems in which better measurement tools and methods could contribute to improved outcomes and reproducibility: instrument calibration and characterization, lysate preparation, DNA template preparation, and protein production.

Regarding the first area, instruments should be calibrated and characterized to understand bias and sources of uncertainty in measured quantities. Instruments common to cell-free workflows that require calibration and characterization include, for example, scales, pipettors, incubators, shakers, plate readers, sonicators, homogenizers, spectrophotometers, and fluorometers. Measurands for this process may include temperature, shaking speed, illumination intensity, energy transferred to the sample, humidity, oxygen concentration,

[3] Certain commercial equipment, instruments, or materials are identified to adequately specify experimental procedures. Such identification implies neither recommendation nor endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

carbon dioxide concentration, and the variation of these across the area or volume in the tool relevant to the sample. In one example, temperature differences across a plate may affect measurements of a standard solution of green fluorescent protein (GFP), and temperature gradients across an individual well may also be significant. To mitigate this, wells containing temperature sensitive indicators, such as cobalt chloride, could be used to measure temperature and temperature uniformity across a plate. In a second example, and especially for the small reaction volumes typical of cell-free systems, evaporation across a plate could be characterized over the course of the reaction. Unfortunately, not every measurement need has an accessible commercial off-the-shelf solution. While many options exist for calibration plates for temperature, humidity, absorbance, and fluorescence for plate readers, the authors are unaware of a test plate with more than one accelerometer to measure shaking at more than one location across a plate. In a third example, measurements of optical density of cell cultures are a routine measurement included in protocols for lysate preparation, but measurements of optical density are neither quantitative [22] nor comparable across instruments. In a final example, including multiple fluorescent calibration curves on the plate could aid in comparability of fluorescence data across time, operators, sites, and equipment. The authors found sampling the calibration curve in many locations beneficial for accounting for variability across the plate of fluorescent measurements during cell-free protein production.

Measurement needs for the second area of lysate preparation are driven by a desire to know the amount and activity of every component of a cell-free reaction. This desire is motivated partly by the belief and hope that such comprehensive characterization will result in better predictive models, more reproducibility and control, and fully rational design for applications of cell-free systems, as well as significantly broaden our fundamental understanding of biology. This reductionist bias overlooks the biological complexity still present in cell-free systems. The mismatch between a fully realized reductionist understanding of cell-free systems and the measured, experimental reality may serve as a means to study the “aliveness” of these and, ultimately, cell-based systems, perhaps even enabling synthetic and minimal cells [5].

Measurements to adequately characterize the components of cell extracts, the energy buffer, and the fully assembled lysates prior to the addition of the DNA template could inform sources of variability. Measurement needs for the cell extract include, for example, a starting strain that has been validated with proteomics and metabolomics, determining the quantity and quality of residual native genomic DNA after lysate preparation, the abundance and activity of endogenous proteins, and the concentration of components in the extract compared to in vivo. On the topic of cell lysis using sonicators and homogenizers, Working Group participants offered anecdotal evidence suggesting that sonicators yield lysates with more reproducible cell-free reactions than other approaches, despite the challenge of calibrating the energy deposited into a sample during sonication. Because vendors of sonication equipment typically report energy output as energy at the piezoelectric element rather than at the probe tip, the sample is exposed to an unknown amount and distribution of energy across the sample. Working Group participants suggested characterizing commercial sonicators using a suspension of beads, dispersed at various sonication settings, and evaluated for sample opacity using well-calibrated plate readers or spectrophotometers. Alternative methods for cell lysis using French press and homogenizers lead to visible oily

residue in lysates. It is unclear if and how this residue affects the lysate performance of the assembled cell-free reaction. Regarding residual genomic DNA and endogenous proteins, the presence and abundance of native genomic DNA in the cell extract can be confirmed by using gel electrophoresis to separate the genomic DNA from the sample, followed by commercially available kits to extract the DNA from the gel and fluorometry or PCR analysis for quantitation. Similarly, to identify abundance and activity of endogenous proteins, Working Group participants suggested fluorescent thermal shift assays, such as ThermoFluor[4], which incorporate a hydrophobic dye that fluoresces upon binding to molten globules and thermal denaturization intermediates, for high-throughput analyses of protein stability [46].

The measurement needs for the energy buffer supplementing the cell extract include, for example, concentration and purity of component ions (typically magnesium and potassium), amino acids, crowding agents (such as polyethylene glycol (PEG)), cofactors (such as NADH and NAD+), and nucleoside triphosphates (such as ATP, GTP, CTP, and UTP). Although well-established methods exist to characterize the components of the energy buffer, few such analyses are routine in cell-free applications. For example, high performance liquid chromatography (HPLC) or capillary electrophoresis [47] can identify and quantitate ions and amino acids. Commercial assay kits are available for high-throughput measurements of the amount of PEG in biological samples [48, 49][4], as well as common cofactors, such as NADH and NAD+ [50-52][4], with colorimetric, fluorometric, or bioluminescent readouts. Nucleoside triphosphates can be analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), hydrophilic interaction liquid chromatography (HILIC), and liquid chromatography–triple quadrupole tandem mass spectrometry with hydrophilic liquid interaction chromatography (LC-TQ-MS-HILIC), which was developed recently for use with cells [53].

Working group participants expressed the strong desire for similar knowledge and characterization of their laboratory prepared lysates as for the PURExpress system. This includes for example, quantifying transcription rates, translation rates, DNA replication rates, and lysate concentration as compared to in vivo. Many Working Group participants suggested performing time course proteomics, metabolomics, and LC-MS/MS of a cell-free reaction, while simultaneously admitting to the prohibitive cost, time, and difficulty in interpreting the results. Fluorescent aptamers, such as malachite green, are used routinely to measure RNA production and decay rates, but anecdotal evidence points to poor performance and degradation in cell-free systems. Alternatively, whole transcriptome shotgun sequencing (RNA-Seq) can be used to determine RNA quantity, screen for novel transcripts, and analyze transcript structure. Molecular beacons [54] currently used to image RNA molecules in real-time in living systems could also be adapted for cell-free systems. Ribosome profiling (Ribo-Seq) techniques [55], such as RiboLace [56], offer positional information about ribosomes flowing along the transcript and can characterize translation. These measurements are rarely performed in cell-free applications and remain unpublished. To characterize and troubleshoot overall cell-free performance, Working Group participants suggested standardized plasmids to comparably monitor and track lysate performance. These plasmids would produce only a

[4]Certain commercial equipment, instruments, or materials are identified to adequately specify experimental procedures. Such identification implies neither recommendation nor endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

single specific known output, such as a transcript, a transcription factor, an orthogonal promoter, or an enzyme, such as kinases and phosphatases, and would include the promotor(s) appropriate for use with the intended organism(s).

Discussion regarding measurement needs for the third area of DNA template preparation focused on the desire for high quality, high quantity, reproducible, and robust DNA templates. Ideally, this would also be achievable for both circular and linear DNA templates and with high-throughput. Existing protocols could, in principle, be expanded to include automation methods, improve reproducibility and throughput, while lowering the cost of DNA template preparation in some cases [57]. DNA templates, which often contain an origin of replication, and any residual native genomic DNA may also replicate in a cell-free reaction, and DNA replication or interactions between DNA and other components of a cell-free reaction may introduce unwanted effects, such as unanticipated resource consumption, that could decrease protein yield or confound reproducibility. These effects could be detected using fluorescent labels or quantitative polymerase chain reaction (qPCR) and may be compatible with high-throughput screening [58]. The Working Group missed the opportunity to define metrics or other criteria related to DNA preparation; measurement methods and needs would have been implicit in such metrics.

For the fourth area of protein production, Working Group participants focused on several ways to potentially improve reproducibility. Accurate and reproducible methods to quantify low concentrations of protein are needed, because plate readers have prohibitively poor sensitivity to low fluorescence signals. This necessitates the use of DNA templates with so-called “strong” promoters to produce enough product to obtain a detectable signal in the cell-free reaction. Additionally, measurements of common fluorescent reporters, such as various green fluorescent proteins, are not reproducible at low concentrations, require accurate instrument calibration, and may not be reproducible across instruments, sites, and operators. Alternative reporters are desirable, including brighter fluorescent reporters with faster folding, alternative colorimetric readouts, and nonfluorescent techniques with high specificity, accuracy, and resolution. Ideally, these would each also be optimized for use in cell-free applications. More direct measurements are needed than those relying on fluorescent reporters. Existing alternatives could include acoustic mass spectrometry [59], cryo-electron microscopy, and chip-based systems coupled to single molecule fluorescence, which remain under development and are not readily scalable. While these methods are prohibitively expensive and time consuming, they may prove useful for visualizing biomolecular conformation.

Working Group participants agreed that a tangible path forward towards more complete and quantitative characterization of a cell-free reaction would be the development of a standard 96-well test plate. The test plate could characterize the cell extract, energy buffer, and cell-free lysate, including initial and final composition. The test plate could also be used to measure the abundance of native genomic DNA and endogenous proteins in the raw cell extract, as well as rates of transcription, translation, and decay. Different chemical and physical conditions could be examined, along with time-course information throughout the duration of a cell-free reaction. In aggregate, this comprehensive information could aid in identifying sources of variability and optimizing reproducibility and performance. A protocol to execute such a test plate could be developed in partnership between NIST and the community of cell-free researchers and include integrated automation. The results of the test

plate could provide an internal check on the quality and performance of a cell-free reaction, offer a means to quantitatively compare results across experiments and laboratories, and perhaps even be reported routinely in publications relying on data from cell-free systems. In this way, laboratory-made cell-free systems could also be compared to commercial alternatives. The proof of concept design of the test and associated protocol could then be passed to commercial manufacturers for additional optimization, production, and distribution. Ideally, the test plate would be designed to characterize and understand cell-free reactions made using cell lysate with the same or more mechanistic detail currently possible with the PURExpress system.

Automation may improve reproducibility in the preparation of cell lysates and DNA templates and the assembly of cell-free reactions. In general, automation systems can reduce user error and uncertainty associated with pipetting, which can be measured. For example, the Labcyte Echo[5] liquid handlers are most commonly reported in automated protocols for cell-free systems, for their ability to accurately dispense small volumes of liquid [60]. The existing anecdotal evidence for the advantages of automation warrants further investigation.

Working Group participants also considered how industry vendors could better serve the community of researchers using cell-free systems. Suggestions included more complete and quantitative manuals for distribution with commercial kits used for preparing components of the cell-free reaction. For example, kits for preparing DNA templates should specify what exactly is eluted at each step of the protocol, as well as the tendency for that step to shear, degrade, or otherwise result in nonfunctional DNA. Quality control for plasmids supplied by commercial vendors could be improved, to spare researchers the cost and time of verifying the sequence themselves. To improve reproducibility for the preparation of cell lysate, purchased or shared strains could ship with specification sheets or other information relevant to easily and rapidly validating the strain. Commercial cell-free kits could provide detailed information on quality control, lot size, and statistics for expected protein yield. For example, the manual for myTXTL requires updating to include the use of an optimized test plasmid that gives approximately double the protein yield than reported in the supplied manual (unpublished results). Vendors could also provide automation protocols with kits and products, as appropriate. Finally, participants called for an overall 10× reduction in the costs for commercial kits used with cell-free systems, both for DNA template preparation and commercial lysates, including the recombinant PURExpress system.

Interlaboratory studies could help identify opportunities to increase reproducibility, develop standardized and reproducible protocols, and clarify the full potential of cell-free systems. To the best of our knowledge, the triservice interlaboratory study is the largest such study performed to date [25]. A much smaller effort was reported as part of iGEM 2018 [61], with an expanded study to include more teams planned for iGEM 2019 [62, 63]. Both of these studies focused primarily on reproducibility in the endpoint measurement of protein yield after completion of the cell-free reaction. Separate studies could optimize the conditions for DNA template extraction, purification, handling, and storage. These results could inform interlaboratory collaborations focused on developing and testing standardized protocols for

[5] Certain commercial equipment, instruments, or materials are identified to adequately specify experimental procedures. Such identification implies neither recommendation nor endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

both circular and linear DNA. NIST is assessing the feasibility and impact of a potential study in this area.

As uses of cell-free systems mature, it is appropriate to engage in rigorous, systematic, and quantitative studies to explore issues around measurement assurance and measurement science for these systems. For example, it remains unclear what degree reproducibility, as well as the number of biological and technical replicates, is required or desirable for cell-free systems. The consensus urges for more reproducibility and more measurements than we have now. Each experiment and measurement must ultimately be fit for purpose and settling these issues will depend upon the intended use of the information gained from each measurement of a cell-free reaction for each application. While cell-free systems are inherently different from cell-based systems, many of the related challenges in reproducibility and measurement assurance are also relevant to biological systems beyond cell-free systems. An open question remains whether and to what extent absolute quantitation, as opposed to the relative quantitation typical of measurements of biological systems, is important to advance reproducibility and applications of cell-free systems.

7. **Sharing and Reporting Information Working Group,** led by Kate Adamala (University of Minnesota)

This Working Group was disbanded, because the topics of sharing and reporting information were drawn extensively through the discussions of the other Working Groups. Instead, William Poole (California Institute of Technology) introduced the BuildACell/CellFree page on OpenWetWare [1], which he administers as a graduate student in the Murray laboratory [4] and is available to researchers in cell-free systems as an initial location to share experimental advice and data.

8. **Findings**

- Cell-free systems generate broad excitement for their potential as an enabling technology platform, but their full capabilities and suitable applications remain unclear.
- A common repertoire of protocols and methods for typical cell-free systems will aid adoption and reproducibility.
- Improved access to data and sharing of information and expertise across laboratories will also aid adoption and reproducibility.
- Improved methods and tools for measuring the components and performance of cell-free systems at all stages in a typical workflow from reagent preparation to final product are needed to advance reproducibility and applications of cell-free systems.
- Cell-free systems may differ from cell-based systems in ways that significantly impact the performance of cell-free systems, when protocols, materials, and measurements for cell-based systems are applied naively to cell-free systems.

9. Recommendations

- Focus the research community and spur investment by identifying specific foundational studies and application areas well-served by cell-free systems.
- Develop and disseminate standard protocols for DNA template and lysate preparation for common use cases.
- Encourage engagement with existing online resources for community and information sharing, such as the BuildACell/CellFree page on OpenWetWare [1].
- Perform interlaboratory studies to identify and test best practices for reproducible lysate and DNA template preparation.
- Develop a standard test plate and protocol to assess reagent quality and facilitate characterization, performance, and reproducibility of cell-free reactions.
- Rigorously assess all assumptions associated with the use of protocols, materials, and measurements for cell-based systems applied to cell-free systems.

10. Outlook

This Report offers practical, actionable recommendations to advance reproducibility in cell-free systems. Applications well-served by cell-free systems will emerge, as the full capabilities of these systems become clearer, in part through more complete understanding of variability and reproducibility. NIST investment and outputs, in close partnership with researchers using cell-free systems, will advance progress in this technology space to support economically-viable applications for bioengineering and biomanufacturing. Cell-free systems may also deepen our fundamental understanding of life itself, for example, by offering an attractive platform towards building artificial and minimal cells [5].

Acknowledgments

The authors gratefully acknowledge the generous support of the J. Craig Venter Institute for access to their facility and support staff, the Build-a-Cell community for inviting the co-location of our Workshops and encouraging participation in both, and illumina, Oxford Nanopore Technologies, QIAGEN, and VectorBuilder for sponsorship. The authors extend warm thanks to the Working Group leaders, as well as to John Glass and Kate Adamala, for donating their time, energy, and resources.

References

[1] OpenWetWare contributors (2019) *BuildACell/CellFree - OpenWetWare* (OpenWetWare). Available at https://openwetware.org/mediawiki/index.php?title=BuildACell/CellFree&oldid=1059033.

[2] Lux M (7 February 2019) Personal communication.

[3] Liu W-Q, Zhang L, Chen M, Li J (2019) Cell-free protein synthesis: Recent advances in bacterial extract sources and expanded applications. *Biochemical Engineering Journal* 141:182-189. https://doi.org/10.1016/j.bej.2018.10.023

[4] Murray RM (2019) *Murray Wiki*. Available at http://www.cds.caltech.edu/~murray/wiki/Main_Page.
[5] Anonymous (2019) *Build-A-Cell*. Available at http://buildacell.io/.
[6] Strychalski EA , Romantseva E (2019) *NIST CELL-FREE Workshop 2019*. Available at https://drive.google.com/drive/folders/14ZAJD9wxwFyq45snUxs3sLMQ2xlAdW7w.
[7] New England Biolabs (2019) *PURExpress® In Vitro Protein Synthesis Kit* Available at https://www.neb.com/products/e6800-purexpress-invitro-protein-synthesis-kit#Product%20Information.
[8] Gyorgy A , Murray RM (2016) Quantifying resource competition and its effects in the TXTL system. *IEEE 55th Conference on Decision and Control*, (IEEE).
[9] Karzbrun E, Shin J, Bar-Ziv RH, Noireaux V (2011) Coarse-grained dynamics of protein synthesis in a cell-free system. *Phys Rev Lett* 106(4):048104. https://doi.org/10.1103/PhysRevLett.106.048104
[10] Siegal-Gaskins D, Noireaux V, Murray RM (2013) Biomolecular resource utilization in elementary cell-free gene circuits. *American Control Conference*, (Washington, DC, USA).
[11] Hu CY, Varner JD, Lucks JB (2015) Generating Effective Models and Parameters for RNA Genetic Circuits. *ACS Synth Biol* 4(8):914-926. https://doi.org/10.1021/acssynbio.5b00077
[12] Mavelli F, Marangoni R, Stano P (2015) A Simple Protein Synthesis Model for the PURE System Operation. *Bulletin of mathematical biology* 77(6):1185-1212. https://doi.org/10.1007/s11538-015-0082-8
[13] Pandey A (2018) Sub-SBML: A Subsystem Interaction Modeling Python Toolbox for SBML Models. *COMBINE 2018*.
[14] Smith L, König M, Waltemath D, Sauro H, Garny A (2017) SED-ML: The Simulation Experiment Description Markup Language.
[15] COMBINE Coordination Board (2019) *COmputational Modeling in BIology NEtwork (COMBINE)*. Available at http://co.mbine.org/home.
[16] Hucka M (2017) *The Systems Biology Markup Language (SBML)*. Available at http://sbml.org/Main_Page.
[17] Sun Z (2019) *Tierra Biosciences*. Available at https://tierrabiosciences.com/.
[18] Kwon YC , Jewett MC (2015) High-throughput preparation methods of crude extract for robust cell-free protein synthesis. *Sci Rep* 5:8663. https://doi.org/10.1038/srep08663
[19] Shrestha P, Holland TM, Bundy BC (2012) Streamlined extract preparation for Escherichia coli-based cell-free protein synthesis by sonication or bead vortex mixing. *Biotechniques* 53(3):163-174. https://doi.org/10.2144/0000113924
[20] Liu DV, Zawada JF, Swartz JR (2005) Streamlining Escherichia Coli S30 Extract Preparation for Economical Cell-Free Protein Synthesis. *Biotechnol Prog* 21:460-465.
[21] Failmezger J, Rauter M, Nitschel R, Kraml M, Siemann-Herzberg M (2017) Cell-free protein synthesis from non-growing, stressed Escherichia coli. *Sci Rep* 7(1):16524. https://doi.org/10.1038/s41598-017-16767-7
[22] Stevenson K, McVey AF, Clark IBN, Swain PS, Pilizota T (2016) General calibration of microbial growth in microplate readers. *Sci Rep* 6:38828. https://doi.org/10.1038/srep38828

[23] Canelas AB, Harrison N, Fazio A, Zhang J, Pitkanen JP, van den Brink J, Bakker BM, Bogner L, Bouwman J, Castrillo JI, Cankorur A, Chumnanpuen P, Daran-Lapujade P, Dikicioglu D, van Eunen K, Ewald JC, Heijnen JJ, Kirdar B, Mattila I, Mensonides FI, Niebel A, Penttila M, Pronk JT, Reuss M, Salusjarvi L, Sauer U, Sherman D, Siemann-Herzberg M, Westerhoff H, de Winde J, Petranovic D, Oliver SG, Workman CT, Zamboni N, Nielsen J (2010) Integrated multilaboratory systems biology reveals differences in protein metabolism between two reference yeast strains. *Nat Commun* 1:145. https://doi.org/10.1038/ncomms1150

[24] Piper MD, Daran-Lapujade P, Bro C, Regenberg B, Knudsen S, Nielsen J, Pronk JT (2002) Reproducibility of oligonucleotide microarray transcriptome analyses. An interlaboratory comparison using chemostat cultures of Saccharomyces cerevisiae. *J Biol Chem* 277(40):37001-37008. https://doi.org/10.1074/jbc.M204490200

[25] Cole SD, Beabout K, Turner KB, Smith ZK, Funk VL, Harbaugh SV, Liem AT, Roth PA, Geier BA, Emanuel PA, Walper SA, Chavez JL, Lux MW (2019) Quantification of Interlaboratory Cell-Free Protein Synthesis Variability. *ACS Synth Biol* 8(9):2080-2091. https://doi.org/10.1021/acssynbio.9b00178

[26] Sun ZZ, Hayes CA, Shin J, Caschera F, Murray RM, Noireaux V (2013) Protocols for implementing an Escherichia coli based TX-TL cell-free expression system for synthetic biology. *J Vis Exp* (79):e50762. https://doi.org/10.3791/50762

[27] Didovyk A, Tonooka T, Tsimring L, Hasty J (2017) Rapid and Scalable Preparation of Bacterial Lysates for Cell-Free Gene Expression. *ACS Synth Biol* 6(12):2198-2208. https://doi.org/10.1021/acssynbio.7b00253

[28] Levine MZ, Gregorio NE, Jewett MC, Watts KR, Oza JP (2019) Escherichia coli-Based Cell-Free Protein Synthesis: Protocols for a robust, flexible, and accessible platform technology. *J Vis Exp* (144). https://doi.org/10.3791/58882

[29] Arbor Biosciences (2017) *myTXTL® – Sigma 70 Master Mix Kit* Available at https://arborbiosci.com/products/mytxtl-sigma-70-master-mix-kit/.

[30] Vallone P (March 15, 2019) Personal communication.

[31] ThermoFisher Scientific (2019) *NanoDrop™ 2000/2000c Spectrophotometers*. Available at https://www.thermofisher.com/order/catalog/product/ND2000LAPTOP?SID=srch-srp-ND2000LAPTOP.

[32] ThermoFisher Scientific (Qubit Fluorometric Quantitation.

[33] Morrow J , Olson N (2012) DNA extract characterization process for microbial detection methods development and validation. *BMC Research Notes* 5:668-681.

[34] National Institute of Standards and Technology (2018) *SRM 2372a - Human DNA Quantitation Standard*. Available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2372a.

[35] Zhang M, Zhang Y, Scheuring CF, Wu CC, Dong JJ, Zhang HB (2012) Preparation of megabase-sized DNA from a variety of organisms using the nuclei method for advanced genomics research. *Nat Protoc* 7(3):467-478. https://doi.org/10.1038/nprot.2011.455

[36] Thermofisher Scientific (2019) *Plasmid DNA Maxiprep Kits*. Available at https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/plasmid-isolation/plasmid-dna-maxiprep.html.

[37] Thermofisher Scientific (2019) *Plasmid DNA Midiprep Kits* Available at https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/plasmid-isolation/plasmid-dna-midiprep.html.
[38] QIAGEN (2019) *Plasmid DNA Purification Kit: QIAGEN Plasmid Kits*. Available at https://www.qiagen.com/us/shop/sample-technologies/dna/plasmid-dna/qiagen-plasmid-kits/#orderinginformation.
[39] Thermofisher Scientific (2019) *Plasmid DNA Miniprep Kits* Available at https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/plasmid-isolation/plasmid-dna-mini-prep.html.
[40] Anonymous (2018) *Noireaux Lab*. Available at http://www.noireauxlab.org/index.html.
[41] Marshall R , Noireaux V (2018) Synthetic Biology with an All *E. coli* TXTL System: Quantitative Characterization of Regulatory Elements and Gene Circuits. *Synthetic Biology: Methods and Protocols*:61-93.
[42] Garenne D , Noireaux V (2018) Cell-free transcription-translation: engineering biology from the nanometer to the millimeter scale. *Curr Opin Biotechnol* 58:19-27. https://doi.org/10.1016/j.copbio.2018.10.007
[43] Smith GR (2012) How RecBCD enzyme and Chi promote DNA break repair and recombination: a molecular biologist's view. *Microbiol Mol Biol Rev* 76(2):217-228. https://doi.org/10.1128/MMBR.05026-11
[44] Sun ZZ, Yeung E, Hayes CA, Noireaux V, Murray RM (2014) Linear DNA for rapid prototyping of synthetic biological circuits in an Escherichia coli based TX-TL cell-free system. *ACS Synth Biol* 3(6):387-397. https://doi.org/10.1021/sb400131a
[45] Marshall R, Maxwell CS, Collins SP, Beisel CL, Noireaux V (2017) Short DNA containing chi sites enhances DNA stability and gene expression in E. coli cell-free transcription-translation systems. *Biotechnol Bioeng* 114(9):2137-2141. https://doi.org/10.1002/bit.26333
[46] Lavinder JJ, Hari SB, Sullivan BJ, Magliery TJ (2009) High-Throughput Thermal Scanning: A General, Rapid Dye-Binding Thermal Shift Screen for Protein Engineering. *J Am Chem Soc* 131(11):3794-3795.
[47] Zunic GD, Spasic S, Jelic-Ivanovic Z (2012) Capillary electrophoresis of free amino acids in physiological fluids without derivatization employing direct or indirect absorbance detection. *Methods Mol Biol* 828:243-254. https://doi.org/10.1007/978-1-61779-445-2_19
[48] Enzo Life Sciences Inc. (2019) *PEGylated protein ELISA kit*. Available at http://www.enzolifesciences.com/ADI-900-213/?gclid=CjwKCAjwqLblBRBYEiwAV3pCJvTNdne9jEzKVXhVYPFgQ7XfyEqsDoHvu0LfP7Ch7mKxEwxFnIPo4xoCg-0QAvD_BwE.
[49] Life Diagnostics Inc. (2017) *PEG SPARCL™ Kits*. Available at http://lifediagnostics.com/peg-reagents/peg-sparcl-kits/?gclid=CjwKCAjwqLblBRBYEiwAV3pCJn84hUEQ1wXWGxHA6oz8S_UajJAC9ghCZ8cxkWnfnNWMmsXN5TMPMxoC6_kQAvD_BwE.
[50] Abnova Corporation (2019) *NAD+/NADH Assay Kit*. Available at http://www.abnova.com/products/products_detail.asp?catalog_id=KA1657.
[51] Cell Bio-Labs Inc. (2019) Title.

[52] Promega Corporation (2019) *NAD/NADH-Glo™ and NADP/NADPH-Glo™ Assays*. Available at https://www.promega.com/products/energy-metabolism/nucleotide-and-co-factor-detection-assays/nicotinamide-adenine-dinucleotide-cell-based-assays/?catNum=G9071.
[53] Matsuda S , Kasahara T (2018) Simultaneous and absolute quantification of nucleoside triphosphates using liquid chromatography-triple quadrupole tandem mass spectrometry. *Genes Environ* 40:13. https://doi.org/10.1186/s41021-018-0101-8
[54] Monroy-Contreras R , Vaca L (2011) Molecular beacons: powerful tools for imaging RNA in living cells. *J Nucleic Acids* 2011:741723. https://doi.org/10.4061/2011/741723
[55] Brar GA , Weissman JS (2015) Ribosome profiling reveals the what, when, where and how of protein synthesis. *Nat Rev Mol Cell Biol* 16(11):651-664. https://doi.org/10.1038/nrm4069
[56] Clamer M, Tebaldi T, Lauria F, Bernabo P, Gomez-Biagi RF, Marchioretto M, Kandala DT, Minati L, Perenthaler E, Gubert D, Pasquardini L, Guella G, Groen EJN, Gillingwater TH, Quattrone A, Viero G (2018) Active Ribosome Profiling with RiboLace. *Cell Rep* 25(4):1097-1108 e1095. https://doi.org/10.1016/j.celrep.2018.09.084
[57] Walsh DI, 3rd, Pavan M, Ortiz L, Wick S, Bobrow J, Guido NJ, Leinicke S, Fu D, Pandit S, Qin L, Carr PA, Densmore D (2019) Standardizing Automated DNA Assembly: Best Practices, Metrics, and Protocols Using Robots. *SLAS Technol*:2472630318825335. https://doi.org/10.1177/2472630318825335
[58] Ranall MV, Gabrielli BG, Gonda TJ (2010) Adaptation and validation of DNA synthesis detection by fluorescent dye derivatization for high-throughput screening. *Biotechniques* 48(5):379-386. https://doi.org/10.2144/000113410
[59] Labcyte Inc. (2019) *Acoustic Mass Spectrometry (MS)*. Available at https://www.labcyte.com/echo-technology/acoustic-mass-spec.
[60] Bailey J, Eggenstein E, Lesnick J (2018) Miniaturization and Rapid Processing of TXTL Reactions Using Acoustic Liquid Handling. (Labcyte Inc.).
[61] Bhatt D (2018) Analysing the variability of cell-free expression systems with an interlab experiment. (iGEM).
[62] Bhatt D (29 October 2018) Personal communication.
[63] Casas A (3 December 2018) Personal communication.

Appendix A: Supplemental Materials

Table 1. Workshop Agenda.

Time	Activity	Room
12:00 pm	Registration (and lunch/coffee/snacks)	Lobby/patio
12:30 pm	Welcome, scope, and deliverables (Elizabeth Strychalski and Jane Romantseva)	Auditorium
1:00 pm	Cell-free wiki (William Poole)	Auditorium

1:15 pm	Breakout sessions: Modeling Lysate preparation DNA template preparation Measurement needs Sharing and reporting information (disbanded)	TBD
3:45 pm 4:00 pm 4:15 pm 4:30 pm 4:45 pm	Reports from breakout sessions: Modeling Lysate preparation DNA template preparation Measurement needs Sharing and reporting information	Auditorium
5:00 pm	Group discussion	Auditorium
5:30 pm	Wrap-up, homework, and concluding remarks (Elizabeth Strychalski and Jane Romantseva)	Auditorium
6:00 pm	Adjourn for joint dinner	Lobby/patio

Table 2. Workshop Participants.

Last Name	**First Name**	**Affiliation**	**Working Group**
Adamala	Kate	University of Minnesota	Lysate preparation
Bashirzadeh	Yashar	University of Michigan	DNA preparation
Benjamin	David	Synlife	Lysate preparation
Buschnyj	Justin	University of Minnesota	Measurement needs
Cole	Stephanie	US Army ECBC	Lysate preparation
Deich	Chris	University of Minnesota	DNA preparation
Ehrenreich	Ian	University of South Carolina	DNA preparation
Garza	Erin	JCVI	Lysate preparation
Gaut	Nathan	University of Minnesota	Lysate preparation
Glass	John	JCVI	Lysate preparation
Heus	Hans	Radboud University	Lysate preparation
Huck	Wilhelm	Radboud University	Modeling
Hutchison	Clyde	JCVI	Modeling
Jackson-Smith	Anton	Stanford University	Measurement needs

Jurado	Zoila	Caltech	DNA preparation
Karas	Bogumil	Western University	DNA preparation
Kempes	Chris	Santa Fe Institute	Modeling
Larsson	Elin	Caltech	Modeling
Lux	Matthew	US Army ECBC	Measurement needs
Maheshwari	Akshay	Stanford University	Measurement needs
Martinez	Heather	Qiagen	DNA preparation
McGovern	Sammi	BioBlaze	DNA preparation
McManus	John	Caltech	Modeling
Merryman	Chuck	JCVI	Modeling
Meyerowitz	Joseph	Caltech	Measurement needs
Moser	Felix	Synlife	Modeling
Murray	Richard	Caltech	Modeling
Nguyen	Michael Truong-Giang	Aarhus University	DNA preparation
Niederholtmeyer	Henrike	UCSD	Lysate preparation
Noireaux	Vincent	University of Minnesota	DNA preparation
Pandey	Ayush	Caltech	Modeling
Panganiban	Jeremy	BioBlaze	Lysate preparation
Poole	William	Caltech	Modeling
Romantseva	Jane	NIST	Measurement needs
Rothschild	Lynn	NASA	Lysate preparation
Shaffer	Jonathan	Qiagen	Measurement needs
Smith	Hamilton	JCVI	DNA preparation
Strychalski	Elizabeth	NIST	Measurement needs
Styczynski	Mark	Georgia Tech	Lysate preparation
Sun	Zachary	Synvitrobio	Lysate preparation
Suzuki	Yo	JCVI	Unknown
Swanson	Haley	San Diego State University	Modeling
Valipour	Michael	Bioblaze	DNA preparation

Walper	Scott	Naval Research Laboratory	Lysate preparation
Wei	Eric	Stanford University	Modeling
Wise	Kim	JCVI	Lysate preparation