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Essential Research Program TRANSFORME Sprint 2020: Developing an Organism Onboarding Pipeline and Establishing Standardization for Army Synthetic Biology

by Randi M. Pullen, Jimmy Gollihar, Madelyn Szilagyi, Katherine Akingbade, and Dimitra Stratis-Cullum

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14. ABSTRACT
Delivering biotechnology to the Warfighter is a DoD priority. The Army needs the agility to rapidly pivot to address its emerging operational needs. Under the U.S. Army's Combat Capabilities Development Command (DEVCOM), ARL invested in building physical/digital infrastructure and a workforce competent in synthetic biology and biotechnology. The ARL Essential Research Program (ERP) Transformational Synthetic Biology for Military Environments (TRANSFORME) was designed to meet the following objectives: 1) harness biology's capacity for custom material production and modification of material properties; 2) develop an agile, modular foundry for key military environments and specialty materials critical to the future operating environment, allowing the United States to out-innovate and counter any adversary; and 3) identify factors that will inform and shape policy, ethics, and approval for the use of synthetic biology products. In 2020, the TRANSFORME ERP designed and conducted a sprint to quickly pivot employee priorities and enhance capabilities while delivering 1) a methodology to rapidly onboard microbial organisms of interest for biotechnology applications and 2) a standard synthetic biology framework enabling the agility to pivot biotechnology capabilities to address emerging operational needs. This report summarizes and analyzes that effort.

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1. Introduction

1.1 Background

Delivering technology to provide Warfighter overmatch by any means necessary is a DoD priority. Biotechnology is the next domain of warfare. The Army needs the agility to rapidly pivot to address its emerging operational needs. Under the U.S. Army's Combat Capabilities Development Command (DEVCOM), Army Research Laboratory (ARL) strategically invested in building a physical/digital infrastructure and a workforce competent in synthetic biology and biotechnology. The ARL Essential Research Program (ERP) Transformational Synthetic Biology for Military Environments (TRANSFORME) was designed to meet the following objectives: 1) harness biology's capacity for custom material production and modification of material properties; 2) develop an agile, modular foundry for key military environments and specialty materials critical to the future operating environment (FOE), allowing the Army to out-innovate and counter any adversary; and 3) identify factors that will inform and shape policy, ethics, and approval for the use of synthetic biology products. In 2020, the TRANSFORME ERP designed and conducted a sprint (Figure 1) with the goal to quickly pivot employee priorities and enhance capabilities while delivering 1) a methodology to address the speed and scale of traditional academic research and rapidly onboard microbial organisms of interest for Army biotechnology applications and 2) a standard synthetic biology framework enabling the agility to pivot biotechnology capabilities to address emerging operational needs. This report summarizes and analyzes that effort.

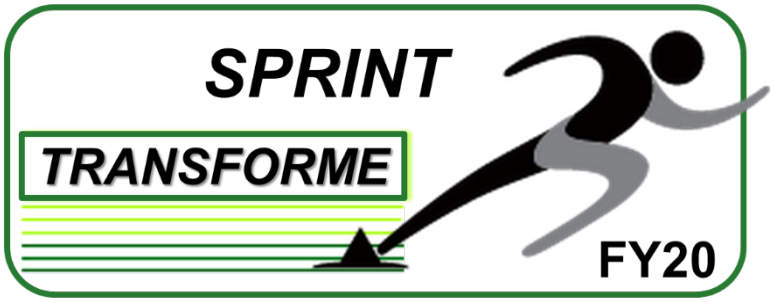

Figure 1. An icon representing the TRANSFORME ERP sprint effort.

1.2 Army Impact Statement

Capabilities in synthetic biology are important for future technologies that will impact the Army of 2040, and beyond. The computational power, dynamic range of function, and novel chemistries of bio and bio-derived products will advance the

state of the art in areas such as materials development, transparent battlefield, and point of need. Foundational research in precision genome engineering, protein engineering, biomanufacturing, and bioinformatics is setting the stage for biotechnology to revolutionize military operations in the future. Figure 2 illustrates that currently there are no products used on the battlefield that include components derived from synthetic biology. The integration of synthetic biology–derived components into formulations is the near-term deliverable. The remarkable complexity of living circuitry opens a realm of future possibilities for temporarily to fully living materials that are able to sense, compute, heal, and transform other materials.

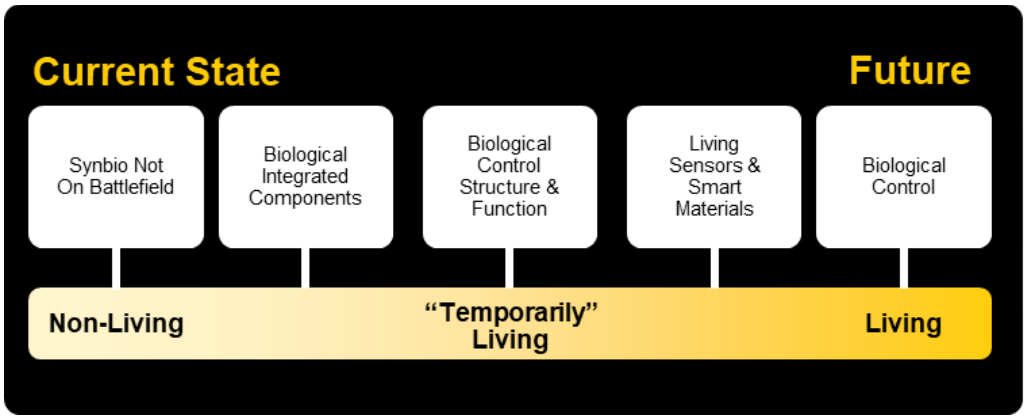

Figure 2. The current and future state of biotechnology for the Army.

1.3 State of ARL Synthetic Biology 2019

ARL sits at the early Technology Readiness Levels (TRLs) and is positioned to partner seamlessly with and act as a bridge between academia and industry (Figure 3). However, ARL is not alone in research and development at early TRLs, since other service labs in the DoD have active programs involving synthetic biology. The DoD Office of the Undersecretary of Defense for Research and Engineering (OUSD R&E) awards funds for Applied Research for the Advancement of S&T Priorities (ARAP). In 2016, the Synthetic Biology for Military Environments (SBME) ARAP was initiated with the aim of creating capabilities and infrastructure across the Army, Navy, and Air Force Research Laboratories for the use of synthetic biology as a key enabler for future defense technology. The major vision of SBME was to establish DoD-wide multifunctional synthetic biology capabilities to develop tools and systems tailored for defense needs such as materials production, environmental sensing, and optimizing warfighter cognitive and physical performance.

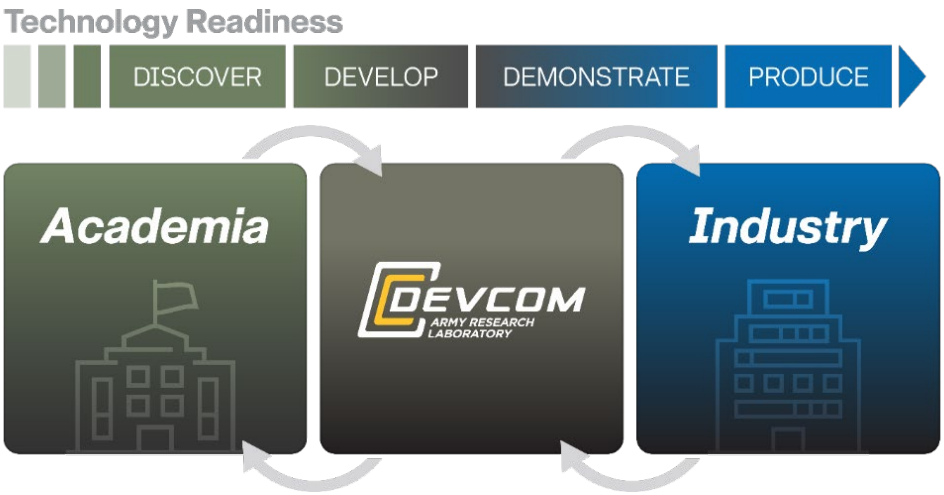

Figure 3. ARL partners with academia and industry to transition products along technology development pipelines leading to higher technology readiness levels. ARL is positioned to develop and demonstrate technologies to serve DoD needs, transitioning in technologies from basic research in academia and moving those technologies out to industry partners for production.

Through the course of the ARAP program (2017–2019), the Synthetic Biology Branch at ARL gained five government employees, nine contractors, and expanded its footprint with physical infrastructure and personnel positioned at the Adelphi Laboratory Center, Aberdeen Proving Ground, ARL-South, and ARL-North.[1] This expansion brought onboard new modern capabilities in bioengineering, synthetic biology, and biomaterials. New equipment such as DNA sequencers, mass spectrometers, and upgraded bioreactors contributed to a more modernized synthetic biology laboratory setting.

One of the key capabilities in synthetic biology, DNA sequencing has seen a dramatic decrease in the cost per megabase (1,000 bases) of DNA sequence in the last 20 years, with the most notable drop occurring in 2008 with the invention of next-generation sequencing.[2,3] From 2001 to 2008, the cost of DNA sequencing per megabase followed Moore's law, but the invention of next-generation sequencing caused a massive drop in price. Moore's law is an observation by the computer circuit industry that the number of transistors in an integrated circuit doubles approximately every 2 years. This observation can be applied to a variety of different technologies, including DNA sequencing. If the improvement of a technology matches the pace predicted by Moore's law, it is widely considered to be performing "exceedingly well."[2,3] At the beginning of 2008, the cost per megabase of DNA sequence was $102.13, but by October 2008 it had dropped to $3.81. By the end of 2019, the cost per megabase of DNA had fallen to $0.008.[2,3]

Compared to the cost of DNA sequencing, the cost of DNA synthesis has not decreased as much (Figure 4). In 2017, the cost of DNA synthesis per megabase was $20,000, while DNA sequencing cost just $0.02 per megabase.[4] DNA synthesis has remained more expensive due to the cost of reagents; however, the development of microarray oligonucleotide synthesis has contributed to the decrease in cost seen in Figure 4.[4,5] Column-based oligonucleotide synthesis was the most commonly used method before the microarray oligonucleotide synthesis technology emerged. The microarray synthesis technology uses significantly less reagents than the traditional column-based methods. The cost of oligonucleotide synthesis using the column-based method is $50–$100 per megabase, while synthesis using microarray costs $0.01 to $0.10 per megabase.[5]

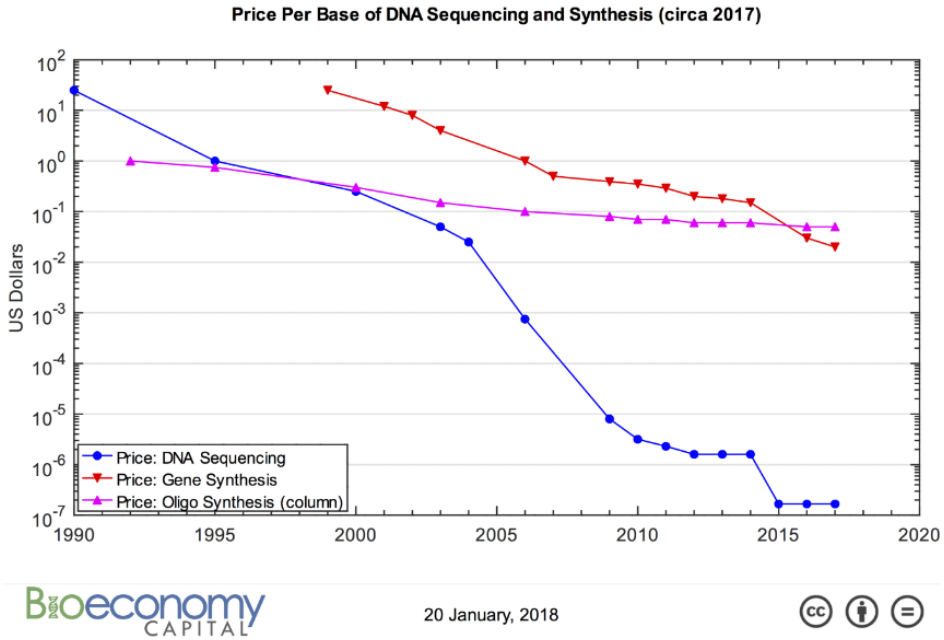

Figure 4. Price per base of DNA sequencing and synthesis: blue represents the price of DNA sequencing per base in U.S. dollars over time; red represents gene synthesis costs per base in U.S. dollars over time; and pink represents column-based oligo synthesis costs in U.S. dollars over time.[4]

Altogether, the field of synthetic biology is rapidly expanding, in part due to the accessibility afforded by decreasing costs in key technologies. The decreasing costs of reading and writing DNA and improvement in predictive models to design complex systems are driving innovation in biotechnology. In April 2025, the National Security Commission on Emerging Biotechnology published a report titled *Charting the Future of Biotechnology*, which outlines the need to prioritize biotechnology at the national level.[6] This report highlights the need to protect and support U.S. biotechnology.

As our nation's first line of defense, it makes perfect sense that the DoD protects and supports biotechnology. Synthetic biology is a priority research area for the Army Futures Command as outlined in the 2019 and 2021 Army Modernization Strategy (Figure 5).[7] As part of the Army Futures Command, ARL surveys the current and future landscape of biotechnology through the lens of national defense and creates programs to address identified needs. Through the implementation of standard synthetic biology frameworks, such as modular cloning, we can further drive down the costs of novel DNA construction through the reuse of parts.

Army Priority Research Areas
1. Disruptive Energetics: Greater than 2x energetic energy over smaller footprints.
2. RF Electronic Materials: Taking advantage of optical and thermal properties of diamond materials.
3. Quantum: Optimized information transfer, sensing, and communication with unparalleled security.
4. Hypersonic Flight: Aerodynamics, materials, and processes.
5. Artificial Intelligence: Increasing speed and agility in which we respond to emerging threats.
6. Autonomy: Maneuverability and off-road mobility of platforms.
7. Synthetic Biology: Reactive and responsive skins/spectrally selective materials/anti-materiel properties.
8. Material by Design: Protection overmatch against future threats.
9. Science of Additive Manufacturing: For next generation munitions for increased range and lethality.

Figure 5. U.S. Army priority research areas.

1.4 Motivation for the TRANSFORME ERP Sprint

Driving down costs and increasing the speed of delivering biotechnology products for the Army was the motivation for the sprint. Under the TRANSFORME ERP, line of effort 1 (LOE1), Synthetic Biology Tools and Chassis Organisms, was given the following mandates:

- Develop an agile, modular foundry for producing specialty materials critical to the FOE, allowing the United States to outpace our adversaries in innovation and application.
- Establish platforms that enable continued agility to pivot to various applications spaces within biotechnology in a secure fashion, thereby meeting the speed of changing operational needs.
- Innovate organism and biomolecule engineering and characterization platforms to deliver streamlined design–build–test–learn for diverse genetic elements, protein architectures, and biological products.

At the inception of the ERP, program leadership assigned a quick-start initiative to pressure test ARL's ability to rapidly pivot the workforce towards the objectives of the new program. This initiative was termed a "sprint" under LOE1. The strategy was to simultaneously address the needs for 1) standardization in synthetic biology practices, 2) employee development, and 3) capabilities that enable agility for other parts of the ERP program. The objective of the sprint was to onboard 11 organisms in 12 weeks. Here the definition of onboard is to "make tractable" or "domesticate," which means that new microbial organisms were to be brought into the lab with the goal of making them tenable for genetic engineering projects, thereby becoming productive towards solving Army problems.

Nature is bountiful in its diversity, complexity, and function. Harnessing the diversity in function across many microbial species gives researchers abundant tools to manufacture and transform materials we use in the modern world. For instance, it is estimated that more than 90% of existing fungal species have yet to be identified.[8] A conservative estimate of approximately 5 million existing fungal species and the current trend of 2,000 new species identified every year means that it will be far beyond our lifetime before they are all identified.[8] However, this represents a vast untapped resource of biochemical reactions that can potentially expand biotechnology capabilities in bioconversion processes. Newly identified strains may have the ability to use complex carbon sources of interest or be adaptive to new processes or environments. With this motivation to expand our access to organisms with unique characteristics, we developed a rapid organism onboarding pipeline.

The method to accomplish this was as follows: 1) create transparency in timelines and deliverables to all levels of performers via Gantt charts, 2) establish weekly technical reporting, 3) establish weekly assignment overviews, 4) adopt and adapt a modular cloning framework and train all users in the framework, 5) convert or "domesticate" all genetic tools into the standard framework, 6) select a diverse mix of organisms to which researchers were completely naïve, 7) synchronize team efforts so that highly analytical data was collected in parallel with fundamental characterization data, 8) create a centralized data repository, 9) demonstrate genetic control and engineering in new organisms, and 10) demonstrate that the collection of methods amassed during the sprint could lead to the accelerated onboarding of diverse microbial organisms.

The 11 organisms selected for the sprint included Gram positive (*Bacillus subtilis* and *Rhodococcus ruber*) and Gram negative bacteria (*Pseudomonas fluorescens, Pseudomonas chlororaphis, Pseudomonas brenneri, Pseudomonas putida*), unicellular eukaryotic fungi (*Saccharomyces cerevisiae, Kluveromyces marxianus*, and *Pichia pastoris*), and multicellular filamentous fungi (*Trichoderma reseei* and

Aspergillus niger) (Figure 6). The specific organisms included a mix of model and non-model organisms. Some organisms had modular cloning toolkits available at the time of the sprint (*S. cerevisiae, K. marxianus,* and *B. subtilis*), and others had toolkits that were published afterwards, by us or others. **Prior to the sprint, none of the researchers had experience working with any of the selected organisms.**

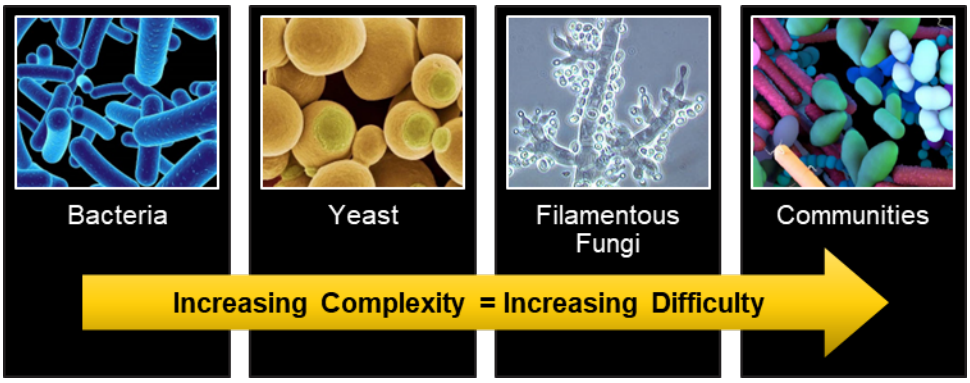

Figure 6. Complexity of microorganisms. Bacteria are single-celled simple organisms. Yeast are eukaryotic organisms that are unicellular but have cellular compartments called organelles. Filamentous fungi are multicellular eukaryotic organisms that form complex networks. In nature, microbes exist in communities with dynamic phenotypes attributed to the complex network of communication and interactions in the microenvironment. From left to right, engineering each of these groups has its own challenges, but is generally increasingly difficult as complexity increases.

Pre-sprint lead time was necessary to ensure all consumables and reagents were on hand such that researchers were unencumbered by lack of materials. Lead time also included literature research to pull genetic parts of interest and domesticate them into the standard cloning framework so that no time during the active sprint period was lost waiting on DNA synthesis (1- to 2-week turnaround depending on length and complexity of parts). Lead time also provided teaming coordination so that redundancies were eliminated, and all team members were thoroughly read into the objectives of the sprint. An overview of the approach for the sprint is shown in Tables 1 and 2.

Table 1. Gantt chart plan for tool development. Green denotes lead time. Yellow denotes active sprint work. Bacterial and eukaryotic toolkits would be developed in parallel.

	Week											
	lead time											
Project	1	2	3	4	5	6	7	8	9	10	11	12
Sprint 1 - Synbio Tools												
Tool Development												
Bacterial toolkits												
DNA Synthesis												
Build/Assembly												
Sequence												
Transformation optimization												
Assay development												
Omics												
Parts testing												
Eukaryotic toolkits												
DNA Synthesis												
Build/Assembly												
Sequence												
Transformation optimization												
Assay development												
Omics												
Parts testing												

Table 2. Gantt chart plan for infrastructure. Green denotes lead time. Yellow denotes active sprint work. In addition to wet lab work, building up digital infrastructure is a necessary component to be able to process and analyze data being generated. In addition, new automation equipment required application support (scripts to run on the instrument).

Biological Foundry Infrastructure												
Design/Computation												
LIMS												
Genome assembly & annotation												
TACC Server												
Build												
Echo												
Colony Picker												
Hamilton												
Test												
Proteomics pipeline												
NGS pipeline												
RNAseq pipeline												
Metabolomics pipeline												

1.5 Improving Biotechnology and Biological Sciences Capabilities

In an effort to create harmony across ARL and the DoD, the ARAP SBME program (2017–2019) funded multiple service labs over 3 years with a budget of approximately $45 million.[1] While the program enjoyed a number of great scientific outcomes, there was no sustainable solution or standardization of methods that would expedite deliverables through collaboration within the DoD. Across the service labs and across the 2019 version of the ARL Biotechnology Branch, all researchers worked following their own molecular biology techniques. The disparity of techniques, many of which lead to the same result but for which genetic parts are not interchangeable, causes a lag in collaboration, communication, and productivity. This lag is increasingly evident as the sphere of collaboration expands outwards, where collaborators within the same or nearby nodes are more likely to follow similar workflows than collaborators at more distal nodes.

Our challenge at the beginning of TRANSFORME ERP was to create a synthetic biology standard framework for designing, building, and testing engineered microorganisms. The sprint staged the TRANSFORME ERP to have the greatest success in syncing synthetic biology research across various competencies and regional sites of ARL. Standardization and consistency are essential in pushing towards artificial intelligence (AI) and machine learning (ML) capabilities; therefore, establishing standard practices will provide the best foundation for accumulating curated data repositories that can be used to build predictive power in designing biotechnology products.

2. Data

2.1 Manpower Commitment

During the sprint, five employees had 100% time commitment, two employees had roughly 80% time commitment, and two employees had 50% time commitment. In addition, one employee joined the sprint after week 4 and two employees served support/admin roles only at 50% commitment. In total, 12 employees—a combination of civilian and contractor workforce—participated in the sprint. Beyond the sprint, four employees committed 100% time to LOE1, one employee committed 100% time to LOE3, and the remaining seven employees either left ARL or were split between various lines of effort. To meet the strict deliverable timelines for the sprint, seven employees reported accruing credit hours.

2.2 Cost of the Sprint

The total costs for salary was approximately $580,000. Between December 2019 and March 2020, approximately $140,000 was spent in total on consumables and other lab supplies. The breakdown of expenses is shown in Figure 7. In Figure 7, "Sprint Consumables" refers to general labware and reagents, and "other orders placed" refers to one-time purchases, including small equipment and unforeseen needs. The Combined All Consumables pie chart reflects that the majority, or 48%, of consumables expenses were towards genetic parts, including but not limited to microbial strains, plasmids, oligonucleotides, gene fragments, and DNA sequencing, whereas the other 52% was spent on traditional scientific labware and reagents. The breakdown of sequencing expenses (not shown) reflects 91% of sequencing costs were for consumables needed for in-house sequencing. Given the background information that synthesis remains a higher cost per megabase than sequencing, it is worth noting that the reuse of parts through a standard cloning framework reduces DNA synthesis costs up front.

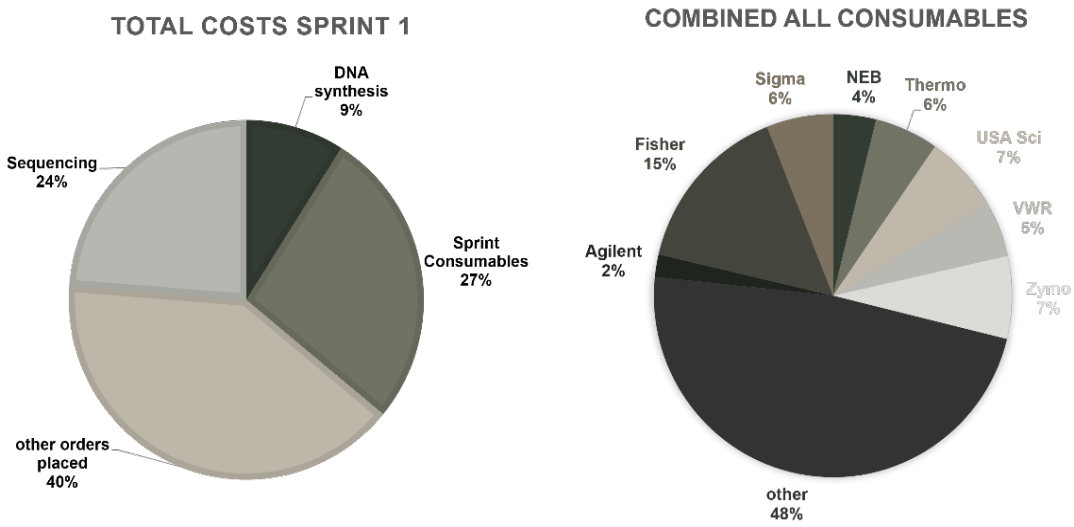

Figure 7. Pie charts of sprint costs. The chart on the left represents the total ~$140,000 spent on labware, equipment, third-party sequencing, and consumables during the sprint. The chart on the right represents the breakdown of sprint consumables from the left-hand chart.

2.3 Deliverables

The pandemic outbreak in 2020 interrupted progress. The final 2 weeks of the planned 12-week sprint were not completed due to ARL limitations, allowing only essential operations. ARL opened many weeks later with restrictions. The restrictions on the number of employees, the number of hours on-site, and the reprioritization of research brought these broader efforts in employee development, capability building, and centralized synthetic biology data repository to near

standstill to focus on remaining program deliverables, including pivoting to COVID support, under the new restrictions. Pandemic restrictions eventually began to ease in the following years and many elements—particularly, the standardization of data and protocols brought to the ERP program during the sprint—remained in place.

A breakout of tasks completed at the time of the COVID shutdown is shown in Table 3. Growth assays refer to culturing the given organism under standard laboratory settings and screening a given number of conditions (temperature, media, nutrient additive, etc.). Fermentation-like growth refers to microbioreactor conditions that are like fermentation conditions typical of large-scale bioproduction. All other types of growth are bench-scale shaking flasks. Long-term storage refers to the cryostocking of an organism and validating the cryostocking can reproduce viable cells. Spot plating refers to the minimal plating volume required to produce colonies that are well isolated and can be selected for outgrowth. Lysis, or breaking apart a cell for nucleic acid extraction, may be more or less difficult depending on the cell wall structure for a given species. Minimal inhibitory concentration refers to the susceptibility of an organism to antibiotics. Transformation is the transfer of DNA into a cell. Growth-alternative carbon source refers to growth in minimal media supplemented with defined carbon sources. Plasmid origin screening refers to identification of self-replicating plasmids that will persist in a strain and support ectopic expression of a gene of interest. Omics samples collected refers to the collection and proper storage of biomass necessary for genomics, transcriptomics, and proteomics preparations. Reporter testing refers to screening the activity of fluorescent, bioluminescent, or colorimetric molecules as readout for expression strength. Integration site testing refers to validating the integration of heterologous cargo (genes of interest) into the organism's genome (instead of expression ectopically such as off a plasmid).

Table 3. Experimental tasks planned for the ERP sprint.

Assay	No. of organisms	No. of constructs or conditions	Total expected	Total completed	Percentage complete (%)
Growth (plate)	11	5	55	55	100
Growth (fermentation-like)	11	5	55	55	100
Long term storage	11	3	33	33	100
Spot plating	11	1	11	11	100
Colony PCR	9	2	18	18	100
Lysis for genomic DNA	11	1	11	11	100
Minimal inhibitory concentration-liquid	11	5	55	50	91
Minimal inhibitory concentration-plate	11	5	55	50	91
Transformation	10	3	30	27	90
Growth-alternative carbon source	6	6	36	30	83
Plasmid origin screening	9	4	36	8	22
Omics samples collected	8	1	192	36	19
Reporter testing	9	5	45	6	13
Integration site test screening	10	3	30	0	0

Key deliverables are quantified in Table 4, representing the point at which the sprint concluded due to the pandemic. Of note, over 650 plasmids were built and verified. In Table 4, melanin strains refers to using various organisms to express a heterologous set of genes leading to the overproduction of melanin. These strains were a product that had a direct transition partner to TRANSFORME ERP LOE2, where extracted melanin could be incorporated into materials for testing. The specific work on melanin-producing strains led to a DoD Biotech Optimized for Operational Solutions and Tactics (BOOST) program and continues to be a biomolecule of interest to the Biotechnology and Biological Sciences (BBS) Division.[9] The onboarding and development of biologically produced melanin for ARL materials formulations began as an initiative under the sprint and LOE1.

Table 4. Sprint deliverables and their completion status at the time the sprint was ended due to the COVID pandemic. Percent completed was calculated by dividing the actual work completed (no. of tasks) by the expected work (no. of tasks).

Product	Completed (%)
Plasmids sequenced	143
Genomes sequenced	100
New organism onboarding pipeline	100
Parts and assemblies	82
Standard operating procedures	75
Technical reports	50
Melanin strains (to LOE2)	22
Other manuscripts	0

2.4 Organism Onboarding Playbook

For the centralization of data generated during the ERP, we set up a shared drive folder location on the ARL server. Access to this location was by permission only. We attempted to set up an architecture that would suit current and future genetic engineering and biotechnology products. With this, all data and information generated during the sprint was deposited in this folder. Altogether, the genetic sequences, strain information, and standard operating procedures comprise our "Organism Onboarding Playbook" (Figure 8). This playbook is a collection of methods and strategies, akin to a recipe book, that would enable researchers to recreate our attempts to onboard new organisms. However, it should be noted that not all organisms are amenable to culturing in isolation or to laboratory conditions, and so it is currently still mostly unpredictable and impossible to successfully onboard all microbes.

As shown in Figure 8, organism husbandry includes the conditions needed to culture the organism in the laboratory. Once it can be propagated reliably and consistently, and be revived from cryostocks, then antibiotic screening and minimal inhibitory concentration assays determine what antibiotics can be used as selectable markers for building genetic constructs. The relevant selectable marker is included on plasmids that are introduced to the cells via various transformation methods. If an organism can be transformed consistently and predictably, then integration into the genome using various methods is attempted. Diverse reporter genes, such as those encoding luminescent or fluorescent proteins, are screened. Reporters can then be used to investigate and characterize promoter and ribosomal binding site (RBS) strength and architecture, leading to a collection of parts with well-characterized expression strength. With the collection of tools in hand, precision genome engineering systems such as clustered regularly interspersed palindromic repeats (CRISPR) can be screened or developed. In addition, secretion tags can be screened for the secretion of heterologously expressed products into the culture. All combined, the organism playbook onboards an organism and builds genetic toolkits that enable a novel microbe to be deployed into various biotechnology product areas.

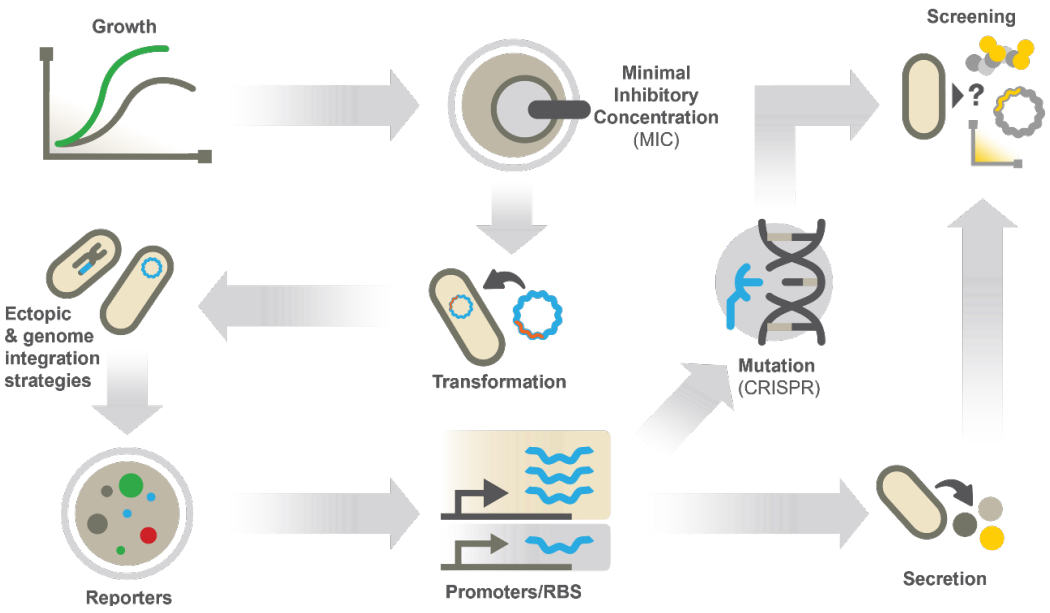

Figure 8. Organism onboarding playbook.

2.5 Contributions to Pandemic Efforts

At the beginning of the pandemic, ARL resources were restricted to only essential functions, and the synthetic biology team was able to contribute to the pandemic response. Using the same standard cloning framework, an engineered version of *S. cerevisiae*, fluorescence-activated cell sorting, and proteomics, ARL helped develop monoclonal antibodies that specifically and strongly bound the SARS-CoV-2 virus spike protein (Figure 9). An extensive collaboration with the medical community moved these novel antibodies to testing for treating severely ill patients. After the pandemic began to wane, ARL returned to its core mission space and the medically relevant work transitioned to Houston Methodist Hospital. This work deserves to be highlighted because in 4 months (pandemic began in January 2020) ARL delivered a product with meaningful societal impact and demonstrated that it was able to rapidly pivot synthetic biology work to address an emergent need using in-house capabilities. The impact of this work, driven by innovations by ARL researchers, is demonstrated by the number of citations listing ARL coauthors (see the Appendix). Publications resulting from continued work (without ARL involvement) are not listed.

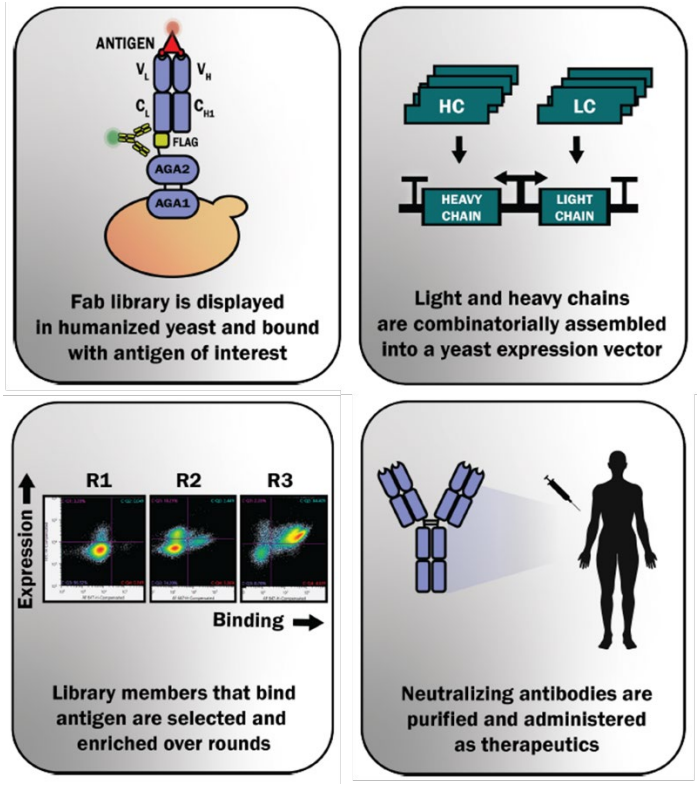

Figure 9. A diagram depicting the synthetic biology tools needed for the building and identification of monoclonal antibodies.

3. Results

3.1 Standardization

A lasting and meaningful impact on ARL Synthetic Biology was accomplished by implementing standardization. While the sprint touched upon standardizing genetic design, data management, and synthetic biology operating procedures, it was the standardized genetic design that had the highest impact (Figure 10). The wider synthetic biology community had already begun creating standard frameworks for cloning (reviewed most recently in Laborda-Mansilla and García-Ruiz[10]) as well as visualizing such information for communication.[11] However, the adoption and implementation of standards is not mandated in order to publish, resulting in a dearth of public (much less machine readable) data that can be used by others. Therefore, the use of standards within the U.S. synthetic biology community is still in its infancy, owing to the lack of means to enforce and reinforce their use. As of 2019, the beginning of the TRANSFORME ERP, ARL synthetic biology researchers had not adopted or practiced synthetic biology standards. As of 2025, that has changed dramatically. ARL is a leader in synthetic biology standardization for the DoD. Through ongoing efforts such as the ARO Synthetic Biology Center

and tri-service collaborations such as the OUSD Tri-Service Biotechnology for Resilient Supply Chain (TBRSC) program, ARL advocates, educates, and propagates the use of Synthetic Biology Open Language (SBOL), SynBioHub, and standard modular cloning practices. Adopting modular cloning standards is essential to leveraging automation platforms, where the interchangeability of parts **increases throughput exponentially and decreases time to development and production**. For instance, one researcher can reasonably set up 96 modular cloning (MoClo) assemblies in 2 h, whereas with automation, 96 times as many assemblies can be set up in the same amount of time (a full 384-well plate every 5 min). The gains in throughput attributed to automation vary at each stage of the modular cloning workflow or at each stage of the Organism Onboarding Playbook. Therefore, the compounding enhancement in throughput and speed depends on the exact workflow being followed.

Through presentations, publications, and briefings, ARL engages in discourse with the broader synthetic biology community on structures for ontologies and data repositories, machine-readable data formats, and creating and refining computational tools that support automation and big data learning. Significant work remains in harmonizing and standardizing data across the DoD. Thankfully, this is being addressed through digital infrastructure prioritization, highlighted through DoD initiatives in building AI/ML capabilities and the OUSD TBRSC programs under the current administration. Our perspective is that once digital infrastructure is in place, adopting and mandating standard practices in synthetic biology design and data repositories across the DoD will be realized.

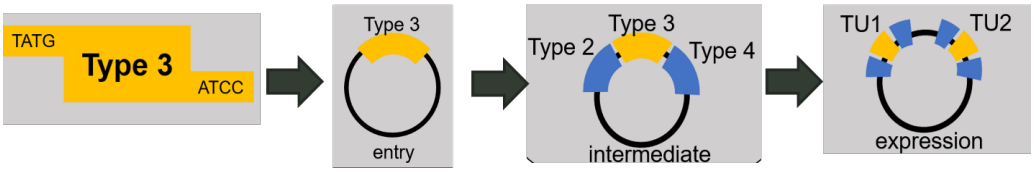

Figure 10. A modular cloning framework. Each type of genetic element (promoter, RBS, cds, terminator, etc.) is designated a fixed "address" or unique 5- and 3-ft overhang that dictates the order in which pieces will assemble (Type 1 followed by 2, followed by 3, etc.). Each part is housed in a cloning vector for storage, deemed the "entry" vector. A one-pot reaction can assemble multiple parts together into intermediate or final expression vectors as long as overhangs are compatible and all positions are filled (e.g., you cannot assemble 1, 2, and 4 without 3).

3.2 Data Repository

The sprint highlighted the need for a centralized data repository. During the sprint, the "Data Vault" was a shared drive on an ARL server that had user-approved read/write accessibility. Once the team was reprioritized to other research efforts, the Data Vault went unused. The ontology used to create the folder architecture of the shared drive location was simplistic and short-sighted, but fit with the available resources. Full-scale initiatives to adapt existing ontologies revealed that no currently existing ontology best suits ARL's needs. Therefore, under the TBRSC program, we are creating a synthetic biology ontology that, at the highest level, captures data and metadata under the headers Design, Build, Test, and Learn. With this better-conceived notion of a Synthetic Biology Ontology, we will be better prepared to collect and curate data for biotechnology development.

With the distribution of Army resources and personnel around the globe, the Army has the capability to provide centralized data and analysis services to its scientific workforce. The DoE is leading the way in this area. The DoD needs to merge efforts with the DoE, either providing additional layers of security or directly adapting what they have already accomplished but under our own security mechanisms and on our own servers. Centralized data repositories and their accessibility across a distributed workforce has yet to be realized in 2025. There are current efforts within the DoD to address this, such as in recent work by the Naval Research Laboratory.[12]

The increasing urgency to improve U.S. biotechnology and biomanufacturing capabilities to outmatch our adversaries, some of whom have been heavily invested in these areas for the better part of two decades, necessitates that we provide the scientific workforce with the ability to generate, access, analyze, and learn from large amounts of data. Machine-readable data and metadata are the keys to unlocking ML from disparate research projects and operations. Accessibility for our distributed scientific workforce is critical to maintaining a strong and capable workforce and attracting new talent. The Army does not need to invent new ways of doing this—industry and the DoE have set the precedent.

3.3 Organism Onboarding

The sprint was able to meet the goal of onboarding 10 organisms in 10 weeks. There were some validation assays and various -omics tasks that did not get completed during the sprint; however, all the genetic parts had been domesticated and assembled in-house, and the biomass samples for the -omics work had been collected. In a few instances, some of these tasks were later completed. In following the Organism Onboarding Playbook, the initial steps are necessary to collect enough information to be able to culture, stock, and transform DNA into the

organism. Once an organism is demonstrated to be competent in taking up DNA in a controllable manner and can be cultured under laboratory conditions, the organism is considered "onboarded." All tasks beyond the first few steps of the playbook are related to testing how DNA is expressed and may be modified in the new organism. Once an organism is demonstrated to have tunable expression of heterologous genes, it can then be explored further as a potential chassis for biotechnology applications. The major deliverable from the sprint was the Organism Onboarding Playbook. The playbook serves as a stepwise process of determining 1) whether a novel organism can be cultured under laboratory conditions, 2) if it is genetically tractable, and 3) the characterization necessary for precision genome engineering and biotechnology applications. The following lists major lessons learned:

- Onboarding new or novel organisms does not require a background in microbiology, and employee development can overcome technical experience gaps.
- -omics is physically and computationally expensive and should serve as the foundation for data repository and data curation standards and requirements.
- Diverse organisms may have diverse culturing requirements, so any facility dedicated to chassis organism development should have appropriate infrastructure and biosafety.
- The playbook is a living document that continues to be expanded and refined, technology is ever-advancing, and new transitions in technology needs to happen continuously if the DoD wants to maintain state-of-the-art capabilities.

Quantifying academic and industry standards in this space is difficult to ascertain from the literature as the domestication of novel organisms for their novel properties in material production was just beginning to be realized.[13]

3.4 Determined Impact to Biotechnology and Biological Sciences Capabilities

Using the predecessor SBME ARAP program for comparison, the ERP accomplished more to standardize synthetic biology and rapidly onboard novel organisms in a shorter amount of time and at a fraction of the cost. The SBME ARAP program had three service labs work with 20 organisms (representing nine different families of bacteria) over the course of 3 years. Of those organisms, ARL worked with five of them. It is important to note the SBME invested significantly in equipment and expanding manpower, which the ERP leveraged.

During the ERP sprint, ARL worked for 10 weeks with 11 organisms (representing three families of bacteria, two families of unicellular fungi, and two families of multicellular filamentous fungi). This effort to rapidly onboard organisms resulted in an 80% improvement over the 4-year ARAP effort: ARAP required an average of 10.4 weeks to onboard one organism, while the sprint accomplished a rate of one organism per week. This is 10 times faster than the ARAP effort; however, we note that some time was spent during ARAP on placing infrastructure and manpower. It should also be noted that since 2020, ARL has worked with approximately 80 different organisms that sit along various phases of the organism onboarding process.

The SBME ARAP's mission was not to provide a framework for DoD researchers to standardize their approaches, though they attempted to develop a platform for a repository to deposit data and a shared software approach; however, issues arose with cyber security and IT allowances. The DoD still does not have a solution for a centralized data repository or data sharing for synthetic biology. Overcoming this obstacle for a single-service lab is a simpler task than at the tri-service level; we created a Data Vault that all members of the effort used to deposit weekly technical reports (metadata), raw and analyzed data, DNA sequences, strain information, safety protocols, standard operating procedures (playbook), and manuscripts in progress. More work is needed to mandate the creation and maintenance of centralized data repositories for ARL efforts, and even more work is needed to have something available at the DoD level. Collaboration is a necessary function to maximize returns on DoD investments, advance biotechnology, and feed innovation. Enabling collaboration (both within the DoD and beyond) via the centralized storage, agile transfer, and analysis of data will advance biotechnology more rapidly to higher technology readiness levels. A deeper discussion on this topic is beyond the scope of this report.

In terms of standardization, the ERP sprint adapted standard modular cloning strategies developed in academia and applied these standard design requirements to all existing and future endeavors. During the sprint, the foundational genetic parts to build toolkits for all 11 organisms were domesticated and were beginning to be validated. Some of these toolkits were published in a technical report[14] and a peer-reviewed journal.[15] Further, the standard toolkit framework was expanded under the recent OUSD TBRSC program to allow for the incorporation of genetic parts from disparate modular cloning frameworks (Hughes, RA, unpublished work). We have propagated these standards across the Army and DoD; most recently, BioMADE MII has adopted the use of our standard design approach. Applying the same standard framework for genetic designs will accelerate biotechnology development by enabling seamless technology transition between

development centers and collaborators, where the end user no longer has to redesign any component for immediate use.

Another benefit to standard design practices is that parts are highly interchangeable, opening up the aperture for interchangeable combinations of genetic parts between diverse organisms. Together with automation and high-throughput screening platforms, this multiplies the diversity of parts that can be screened for any synthetic biology product, increasing productivity by up to ***2 orders of magnitude over manual methods***. At present, the automation facility at ARL-South can build and screen approximately 4,000 colonies, representing approximately 1,000 unique constructs in 1 week. During the sprint, at which time no automated platforms were being used, the total number of constructs built was approximately 600. There is no existing documented metric by which to compare the productivity of the ARAP program in terms of genetic constructs built.

The ARAP was a 4-year, $45 million effort, including salary, with approximately one-third of the effort towards engineering new organisms and a portion of those funds towards infrastructure. The ERP sprint was an approximate $150,000 effort, with approximately $580,000 towards salary for 12 weeks. While we cannot fully make a direct comparison between these two programs due to the diversity and magnitude of work attempted by each, we can conclude that the ERP sprint executed approximately 80% of planned tasks, was 100% successful at onboarding 10 organisms in 10 weeks, and permanently impacted ARL biology and biotechnology capabilities. Many of the subsequent customer-funded projects within ARL leveraged and expanded upon themes first presented during the sprint—namely in standardization, high-throughput genetic engineering, and rapid chassis organism development.

3.5 Lasting Impact

The ERP sprint set the foundation for synthetic biology efforts for the remainder of the TRANSFORME ERP program and expanded the agility of ARL biotechnology capabilities. The lasting impacts can be quantified as discussed in the following subsections.

3.5.1 Publications

In total, 12 ARL publications can be attributed to work completed during the sprint or influenced by work initiated under the sprint (see the "Technical Reports" section of the Appendix). There is no mechanism by which to track the impact of DoD technical reports as there is no database collecting information on how technical reports are cited or used. There have been 22 peer-reviewed journal

articles published since the sprint. The majority (18/22) are related to work done in response to the COVID pandemic. Of those, many have had a high impact on the medical community through high-impact journals and numerous citations (see the "Peer-Reviewed Publications" section of the appendix). This publication record portrays ARL synthetic biology efforts to be highly medically aligned; while medical research is not part of ARL's research portfolio, these publications demonstrate the agility with which BBS synthetic biology work can pivot to meet urgent needs.

3.5.2 Programs Started after the Sprint That Build upon Themes from LOE1

The following ARL customer-funded programs were started after the sprint:

- DoD Biotech Optimized for Operational Solutions and Tactics (BOOST) (2020–2021)
- Office of the Undersecretary of Defense for Research and Engineering (OUSD R&E) Tri-Service Biotechnology for Resilient Supply Chain (TBRSC) (2022–2025)
- OUSD Laboratory University Collaboration Initiative (LUCI) (2022–2025)
- ARL Grassroots Innovation Thinktank (GRIT) (2023–2024)

Themes from ARL's LOE1 sprint have also influenced the design of extramural programs, such as the Defense Advanced Research Projects Agency (DARPA) Tellus and the Army Research Office's Synthetic Biology Center.

3.5.3 Growth of the Biotechnology and Biological Sciences Division

The BBS division has grown from a single branch (Sensors and Electronic Devices Directorate, Biotechnology Branch in 2019) to an entire division comprising three branches. This growth demonstrates ARL's investment and commitment to synthetic biology as well as biomaterials and bioeffects.

3.5.4 Honors, Awards, and Feedback

- The sprint was marked as a top accomplishment by the Biotechnology Branch for FY20
- 2020 ARL Honorary Award (Impactful Teaming)
- 2020 Director's Research Award (DIRA) Early Career Award (Jimmy Gollihar)
- The sprint and the projects that grew out of the sprint (chassis organism development and strain engineering standards) were presented to the

Technical Advisory Board for Biotechnology and Biological Sciences at ARL in July 2024. We received high praise for our efforts as "first in the field" to undertake such a pressure test and successfully demonstrate our capability.[16]

4. Conclusions

Life is an intricate choreography of biological components powered by machinery driven by highly complex circuitry. The diversity of circuitry, components, and machinery that is present across life-forms can be domesticated and repurposed through synthetic biology. The TRANSFORME ERP set the following overarching goal which culminated the efforts of all three LOEs: Demonstrate the use of synthetic biology to accelerate the speed of biotechnology development.

For the 2020 ERP sprint, we planned and executed an effort requiring 12 employees. The major outcomes were the Organism Onboarding Playbook and implementation of synthetic biology standards. In 10 weeks and with a total expenditure of approximately $720,000, we gained engineering capabilities in eight unicellular organisms and began work in two filamentous fungi (otherwise known as mold). These organisms are relevant to biotechnology interests in material decomposition, material formulations, and biomanufacturing. The sprint was halted 2 weeks early due to the pandemic, but the foundation was established for the optimal Organism Onboarding Playbook (Figure 11). While we attempted 10 organisms in parallel during the sprint, we have not followed up to attempt higher throughput. Given the extensive automation infrastructure in place as of 2025, we approximate that 10–20 times more single-celled organisms and two times more multicellular organisms can be pushed through organism onboarding following the prescribed timeline when adequate resources (manpower, in particular) are available. While organism onboarding focuses on domesticating new organisms to be able to engineer them, our current automation infrastructure supports building and screening thousands of unique constructs or engineered strains at a time. From 2019 to 2025, we have increased our throughput in designing, building, and characterizing unique strains by up to three orders of magnitude across a number of efforts within BBS, including microfluidics.

These capabilites enable the DoD to rapidly pivot to synthetic biology projects that meet an immediate need. By implementing standardization and investing in state-of-the-art infrastructure, we have driven down the cost of developing new biotechnology products for the DoD. In addition, we are accumulating data repositories and predictive modeling tools that heighten our capacity to analyze

complex data. Altogether, the sprint began a surge in ARL synthetic biology capabilities that are contributing to the long-term vision of the Army and DoD.

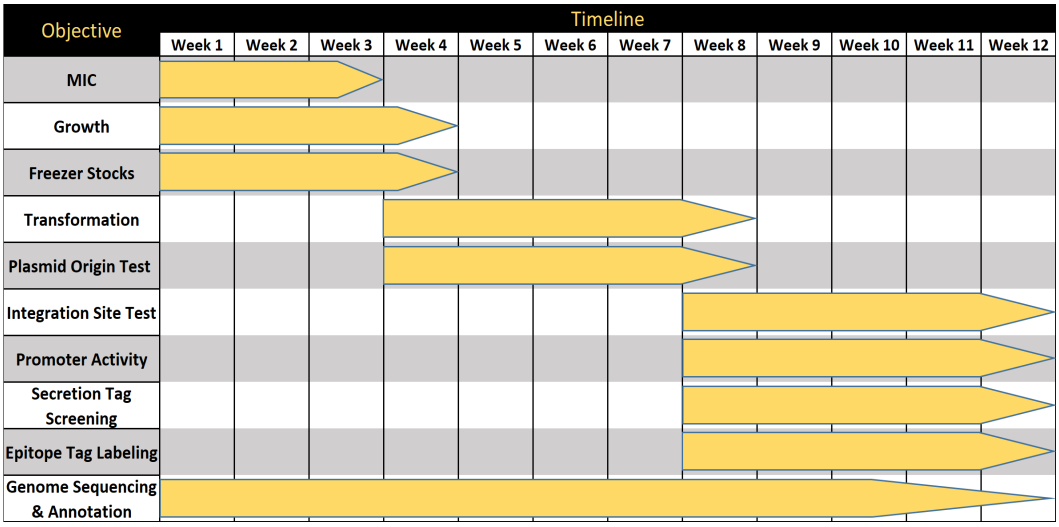

Figure 11. The Organism Onboarding Playbook represented as a Gantt chart.

We found that we can quickly refocus manpower and accelerate our capabilities to meet the Army's needs, which is especially important considering global events such as pandemics. Traditionally, this kind of work has taken years; we showed that we can onboard and begin to engineer undomesticated organisms in a matter of weeks. By focusing team efforts and establishing standardization, we have expanded the possibilities of applications using synthetic biology. We are harvesting molecular machinery and circuitry from nature and applying it to material production for military uses, such as melanin for protective materials. The output materials and knowledge products from our strategic approaches have transitioned across the Army to collaborators at other labs. The TRANSFORME ERP accelerated the Army's synthetic biology capabilities to the new start Living Materials mission line, the only of its kind across the service laboratories, that was pitched in 2016. By expanding the breadth and number of chassis organisms we can engineer, and with the modularity of the engineering toolkits, the Army was able to open the aperture on the production and studies of high value, novel, and complex materials.

5. Future Outlook

To address the needs of our Warfighters, ARL aims to deliver disruptive technologies that provide overmatch. For synthetic biology to contribute to this mission, it is important to note that we do not need to be able to domesticate every

organism we come across, but to have in our arsenal a diverse selection that will function in the designated operating environment. Whether that environment is the operating theater as part of a device, an industrial-scale or point-of-need fermentation tank, or the laboratory applied in new biotechnologies, ARL will ensure that the Army's needs are met by maintaining state-of-the-art synthetic biology capabilities. We are now leveraging the capabilities we built for complex systems integration.

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Appendix. ARL Publications from Work Started in the TRANSFORME ERP Sprint

A.1 ARL Reports

Baumbach J et al. Fungal degradation of organic materials of department of defense interest: a review. DEVCOM Army Research Laboratory (US); 2024. Report No.: ARL-TR-9996.

Halper SM, Chu M, Zu T, Pullen RM, Schwalm ND 3rd. Engineering of Escherichia coli BL21 for melanin production on glucose media using CRISPR/Cas9. DEVCOM Army Research Laboratory (US); 2022. Report No.: ARL-TR-9612.

Halper SM, Renberg RL, Gollihar JD, Schwalm ND 3rd. Promoter libraries for tunable gene expression in Actinobacteria. DEVCOM Army Research Laboratory (US); 2022. Report No.: ARL-TR-9586.

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A.2 Peer-Reviewed Publications

Table A-1 provides data on ARL-coauthored peer-reviewed journal articles published from 2020 to 2025 on topics introduced during the Essential Research Program (ERP) sprint. The list that follows correlates to the far-left column of the table.

Table A-1. Peer-reviewed journal articles from 2020 to 2025 published with ARL coauthors on work and themes introduced during the ERP sprint.

Ref. no.	No. cited by	Journal	Journal impact factor	Publication date
1	21	Science Advances	11.7	Apr. 2020
2	1	Journal of Immunology	3.6	May 2020
3	205	American Journal of Pathology	4.7	Aug. 2020
4	138	Journal of Clinical Investigation	13.3	Sep. 2020
5	76	mBio	6.4	Oct. 2020
6	169	American Journal of Pathology	4.7	Nov. 2020
7	138	Journal of Clinical Investigation	13.3	Dec. 2020
8	107	American Journal of Pathology	4.7	Jan. 2021
9	21	Communications Biology	5.2	Feb. 2021
10	6	MedRxiv	. . .	Mar. 2021
11	14	bioRxiv	. . .	Apr. 2021
12	168	Science	44.7	May 2021
13	33	American Journal of Pathology	4.7	June 2021
14	18	American Journal of Pathology	4.7	Oct. 2021
15	32	Molecular Cell	14.5	Dec. 2021
16	15	Nature Communications	14.7	May 2022
17	5	Communications Biology	5.2	Dec. 2023
18	1	Applied and Environmental Biology	3.9	July 2024
19	1	Briefings in Functional Genomics	2.5	Aug. 2024
20	0	ACS Synthetic Biology	3.8	Jan. 2025

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List of Symbols, Abbreviations, and Acronyms

AI	artificial intelligence
ARAP	Applied Research for the Advancement of S&T Priorities
ARL	Army Research Laboratory
BOOST	Biotech Optimized for Operational Solutions and Tactics
CRISPR	clustered regularly interspersed palindromic repeats
DARPA	Defense Advanced Research Projects Agency
DEVCOM	U.S. Army Combat Capabilities Development Command
DIRA	Director's Research Award
DNA	deoxyribonucleic acid
DoD	Department of Defense
DoE	Department of Energy
ERP	Essential Research Program
FOE	future operating environment
GRIT	Grassroots Innovation Thinktank
LOE	line of effort
LUCI	Laboratory University Collaboration Initiative
MIC	minimal inhibitory concentration
ML	machine learning
PCR	polymerase chain reaction
-omics	shorthand for the analysis of genomes, transcriptomes, proteomes, etc.
OUSD R&E	Office of the Undersecretary of Defense for Research and Engineering
RBS	ribosomal binding site
SBME	Synthetic Biology for Military Environments
S&T	Science and Technology
TBRSC	Tri-service Biotechnology for Resilient Supply Chain
TRANSFORME	TRANSformational synthetic biology FOR Military Environments
TRL	Technology Readiness Level

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