Algal Biology Toolbox Workshop Summary Report

San Diego, California

May 2016

Preface

The U.S. Department of Energy's (DOE's) Office of Energy Efficiency and Renewable Energy (EERE) invests in a diverse portfolio of energy technologies to achieve a stronger economy, a cleaner environment, and a more secure energy future for America. This report summarizes the results of a public workshop sponsored by DOE-EERE in San Diego, California, from May 24–25, 2016.

The views and opinions of the workshop attendees, as summarized in this document, do not necessarily reflect those of the U.S. government or any agency thereof, nor do their employees make any warranty, expressed or implied, or assume any liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represent that its use would not infringe upon privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States government or any agency thereof.

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Introduction

On May 24–25, 2016, DOE-EERE's Bioenergy Technologies Office (BETO) hosted the "Sharpening Our Tools: Algal Biology Toolbox Workshop" in San Diego, California.

BETO works to accelerate the development of a sustainable, cost-competitive, advanced biofuel industry that can strengthen U.S. energy security, environmental quality, and economic vitality, through research, development, and demonstration projects in partnership with industry, academia, and national laboratory partners. BETO's Advanced Algal Systems Program (also called the Algae Program) has a long-term applied research and development (R&D) strategy to increase the yields and lower the costs of algal biofuels. The team works with partners to develop new technologies, to integrate technologies at commercially relevant scales, and to conduct crosscutting analyses to better understand the potential and challenges of the algal biofuels industry. Research has indicated that this industry is capable of producing billions of gallons of renewable diesel, gasoline, and jet fuels annually. R&D activities are integrated with BETO's longstanding effort to accelerate the commercialization of lignocellulosic biofuels.

Several goals and objectives support the long-term strategy of the Advanced Algal Systems Program, which aims to develop and demonstrate technologies that produce sustainable algal biofuel intermediate feedstocks that can perform reliably in conversion processes to yield renewable diesel, jet, and gasoline. BETO has an aggressive productivity target of 2,500 gallons of biofuel intermediate per acre annual average by 2018, and 5,000 gallons by 2022. The goal is to achieve a $3 per gasoline gallon equivalent price point and a minimum of 50% reduction in greenhouse gas emissions compared to gasoline. BETO is incorporating a near-term focus on high-value nutritional and chemical products to enable the technology development necessary for commercialization of cost-competitive algal biofuels by 2030.
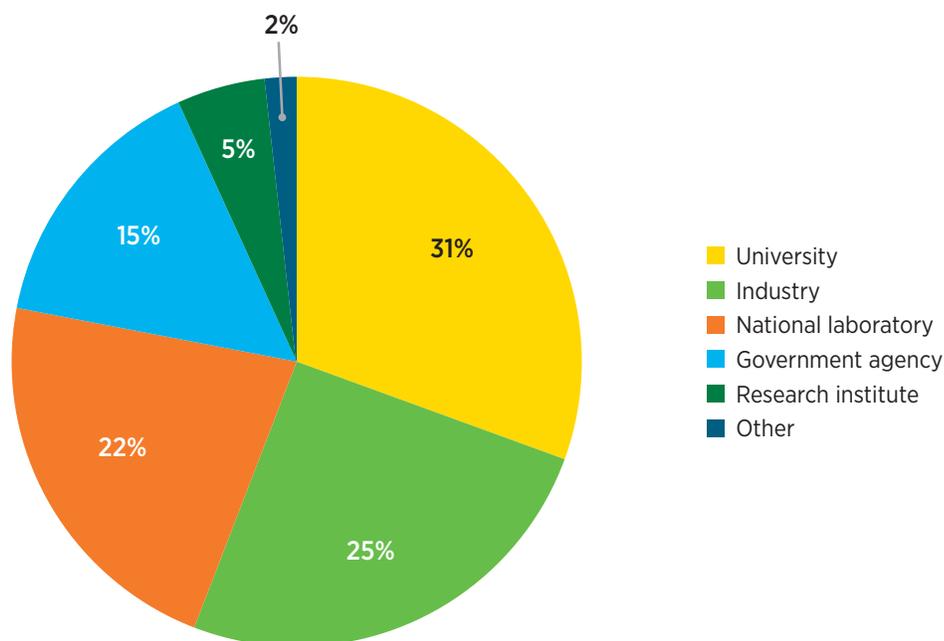
BETO's Advanced Algal Systems Program regularly hosts algal biofuels strategy workshops to help break down critical technical barriers and promote sustainable and affordable algal biofuels.[1] At previous events, stakeholder input established three main points of understanding on which to build future discussions: (1) algal bioproducts should be able to act as building blocks for the future development of algal biofuels, (2) economic viability of the target product should be taken into consideration, and (3) products should be environmentally favorable.[2] This workshop was the first algal biofuels strategy session to focus specifically on improvements in algal biology—a key research focus required to advance the economic viability of algae-based biofuels.

The objective of this event was to discuss R&D needed to achieve affordable, scalable, and sustainable algae-based biofuels. Presentations from algae experts overviewed the tools and resources available to grow, monitor, valorize, and genetically enhance algae for biofuel and bioproduct production. Attendees participated in breakout sessions to provide input about the challenges in algal biology, the metrics of success in algae strain development, and the resources needed to overcome challenges and achieve success. Fifty-eight stakeholders attended the event, representing industry, national laboratories, universities, government agencies, and research institutions (Figure 1).

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[1] "Algal Biofuels Strategy Workshops," U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, accessed June 1, 2016, www.energy.gov/eere/bioenergy/algal-biofuels-strategy-workshops.

[2] Andrea Bailey, G. Jeremy Leong, and Nichole Fitzgerald, Bioproducts to Enable Biofuels Workshop Summary Report, U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office, December 2016, http://energy.gov/sites/prod/files/2015/12/f27/bioproducts_to_enable_biofuels_workshop_report.pdf.

Figure 1. Breakdown by sector of Algal Biology Toolbox Workshop attendees

Legend:
- University — 31%
- Industry — 25%
- National laboratory — 22%
- Government agency — 15%
- Research institute — 5%
- Other — 2%

Workshop Concept and Process

The purpose of the Algal Biology Toolbox Workshop was to collect input from lead experts in the field of algal biology regarding (1) the current state of algal biological tools, including our understanding of algal biology and biochemistry, available molecular toolboxes, omics databases, and other resources; (2) challenges to developing and applying a full suite of biological tools to improve algae performance and system robustness; and (3) strategies to advance progress toward commercial algal biofuels. BETO emphasized a focus on near-term opportunities to advance commercialization of algal biofuels and enable the production of algae-based bioproducts. Attendees were asked to provide input regarding the following questions:

- What are challenges in algal biology, specific to genetic tools, which pose significant hurdles to progress over the next 5 years?

- What does success look like; what attributes would the ideal commercial algae strain(s) possess?

- What tools and resources exist to overcome the challenges to achieve the ideal strain(s)?

  - What tools and resources are still needed?

Responses from stakeholders affiliated with industry, national laboratories, universities, and research institutions will help BETO shape its near-term research funding strategy.

The workshop began with an opening presentation from BETO to provide attendees with necessary context about BETO's mission and the goals of the workshop. Also on the first day of the workshop, several experts in the field of algal biology gave presentations on recent developments in their R&D efforts. On the second day of the event, BETO facilitated breakout discussions that aimed to identify challenges in algal biology and strategies for advancing the field. Breakout session participants provided their input regarding available molecular toolboxes, omics databases, and other algal biology and biochemistry resources. Participants also discussed potential strategies to overcome challenges and to measure the field's progress toward commercial algal biofuels. See appendix A for a detailed agenda.

Summary of Presentations

Experts in the field of algal biology shared their recent successes with event attendees to further the algal biology community's understanding of the current state of the art. The speakers were selected with a goal of ensuring that a range of expertise was represented within the presentations. This section of the workshop summary report provides highlights of each speaker's presentation with a focus on current challenges in algal biology, definitions of success in strain improvement, and the tools and resources needed in order to address near-term challenges to viable commercial algal biofuels and bioproducts.

New Developments in Algal Biology

BETO has hosted several algal biofuels strategy workshops to discuss R&D needed to achieve affordable, scalable, and sustainable algae-based biofuels. This particular event focused more specifically on algal biology, a field critical to the advancement of algal biofuels. As a starting point, an introductory presentation by Dr. Amanda Barry provided a thorough overview of recent developments in biology and biochemistry, as they relate to algae.
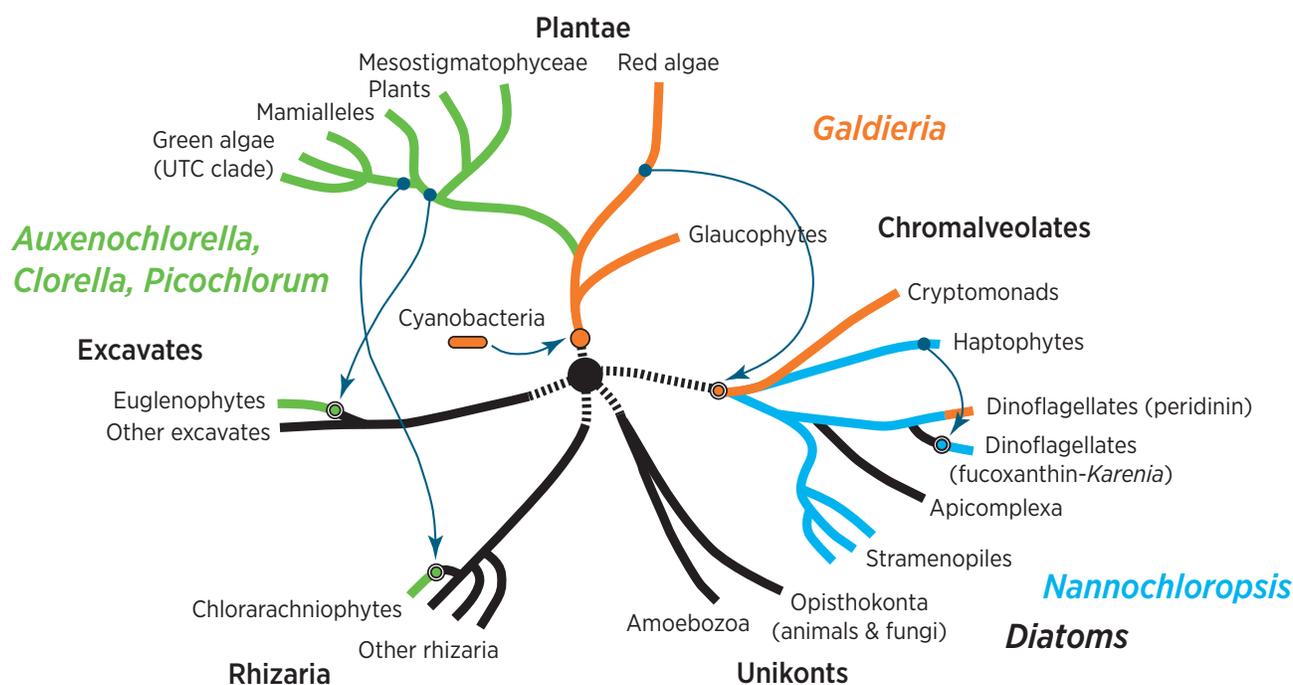
New Discoveries in Biology and Biochemistry
Amanda Barry, Ph.D.
U.S. Department of Energy, Bioenergy Technologies Office, Advanced Algal Systems Program

Dr. Barry, Scientist and Technology Development Manager in the BETO Advanced Algal Systems Program, opened the 2-day Algal Biology Toolbox Workshop with an overview of new discoveries in biology and biochemistry. Her presentation began with a brief overview of the genetic tools for strain optimization, which include directed evolution, heterologous and native gene expression, genome editing, and breeding. Next, she provided an overview of omics approaches and bioinformatics approaches, which include genomics, transcriptomics, proteomics, metabolomics and lipidomics, and phenomics. Dr. Barry explained that the incredible genetic diversity of algae makes it difficult to create tools and methods that are universally applicable, necessitating an extensive suite of genetic tools and omics approaches. Figure 2 below illustrates algae's genetic diversity and, therefore, its diversity of opportunity in commercial development.

Figure 2. Genetic diversity of algae[3]

As an introduction to the current state of research, Dr. Barry stated that gene transfer system development, an important milestone, had been established in many algal strains, but not all. She also differentiated between the statuses of development in different strains. In cyanobacteria, for example, transgenic approaches have enabled the production and secretion of cellulose, sucrose, ethanol, isobutanol, ethylene, and free fatty acids. In eukaryotic strains of microalgae, targeted metabolic engineering to increase the production of lipids has begun in green algae, diatoms, and brown algae.

Dr. Barry also reviewed results from two major public-private partnerships supported by DOE with the goals of advancing algae science and commercializing algal biofuel. One of these partnerships, the National Alliance for Advanced Biofuels and Bioproducts, was successful in developing technologies with the potential to reduce the cost of algae-based biocrude from $240 to $7.50 per gallon. The alliance, led by the Donald Danforth Plant Science Center in St. Louis, Missouri, had over 30 partners, including national laboratories, universities, and companies. The collaborative effort helped to establish a sustainable algal biofuels and bioproducts industry. A final report published in 2014[4] summarized important technical results such as new strain development, improved cultivation, low-energy harvesting technology, and high-yield extraction and conversion technology.

Another public-private partnership, the Consortium for Algal Biofuel Commercialization, recently published its final report.[5] DOE established this group with the goal of enabling commercial viability of algae-based biofuels with a focus on key aspects of algal biofuel production, such as development of genetic tools, crop protection, and nutrient utilization and recycling. The consortium, led by the University of California, San Diego, also developed education programs that have trained more than 200 research scientists and laboratory technicians and have introduced more than 50,000 students worldwide to the benefits of sustainable alternative fuels.

Molecular Toolbox Development and Gene Expression

Molecular toolbox development has been identified as a crucial effort in advancing the field of algal biology. Professor Matt Posewitz with the Colorado School of Mines focuses research efforts at his laboratory on *Nannochloropsis*, *Chlorella*, and cyanobacteria strains. Professor Mark Hildebrand, who runs a research laboratory at the University of California, San Diego, focuses his research efforts on diatoms.

Molecular Tools for Improving Biofuel Phenotypes in Algae and Cyanobacteria
Matthew Posewitz, Ph.D.
Colorado School of Mines

Colorado School of Mines collaborates on research regarding generalized photoautotrophic biofuel schematics. During his presentation, Professor Posewitz reported results from several efforts. One study used carbon partitioning to optimize fatty acid levels, starch accumulation, and photosynthetic performance. Another project involved prospecting for better organisms and better proteins from new ecosystems with various environments. In order to isolate genes that will survive high light and temperature conditions, researchers selected strains found in hot and salty locations.

Another effort focused on developing *Nannochloropsis gaditana* into a model marine, biocommodity production strain. This species is a promising lipid producer, and the strain's cell walls are known for their durability and robustness. Continued work on this species is focusing on improving harvesting efficiency. As part of this effort,

[3] Beverley R. Green, "Chloroplast Genomes of Photosynthetic Eukaryotes," *The Plant Journal* 66 (2011): 34–44, doi:10.1111/j.1365-313X.2011.04541.x.

[4] National Alliance for Advanced Biofuels and Bioproducts, *National Alliance for Advanced Biofuels and Bioproducts Synopsis Final Report*, http://www.energy.gov/eere/bioenergy/downloads/national-alliance-advanced-biofuels-and-bioproducts-synopsis-naabb-final.

[5] Consortium for Algal Biofuel Commercialization, *Consortium for Algal Biofuel Commercialization Final Report*, Dr. Stephen Mayfield (principal investigator), DE-EE0003373, December 4, 2015, http://www.osti.gov/scitech/biblio/1234700-consortium-algal-biofuel-commercialization-cab-comm-final-report.

the Colorado School of Mines laboratory is developing transformation techniques for many algal strains. Professor Posewitz sees several opportunities for future research, including efforts to decrease light harvesting antennae, improve carbon assimilation, tailor carbon products, harvest cells from water, and generate secreted products.

Genetic Manipulation Approaches for Metabolic Engineering of Microalgae
Mark Hildebrand, Ph.D.
University of California, San Diego – Scripps Institution of Oceanography

Dr. Hildebrand began his presentation with a statistic about the power of genetic tools: Since the 1930s, corn yields have increased by 600%. Similarly, he explained, genetic alteration is required in order to impart a stable phenotypic environment that allows for cost-effective production of microalgal strains. With respect to algae, choosing which genes to manipulate is still a challenge. It is clear, he stated, that lipid content and growth rate are the two traits that are major drivers for economical biofuel production.

At this time, genetic engineering is accepted as the preferred approach to genetic manipulation because it is easier to incorporate multiple traits. Limited or undeveloped methodology for preferred species, the importance of using supporting insight from fields of study such as transcriptomics, and the possibility of missing valuable genes all contribute to the complexity of genetic engineering. Given these complexities, mutagenesis and selection approaches are also valuable in improving performance of microalgal strains for cost-effective production. Dr. Hildebrand's laboratory team used a technique called mutagenesis, which involved the engineering of gene mutations and resulted in significantly higher lipid accumulation in 36% of cells screened with no detrimental growth effects.

Dr. Hildebrand noted that genetic manipulation is more readily achievable for some types of microalgae than for others. Two important factors are (1) transformation ability, which is driven by identification of appropriate promoters, and (2) proficiency, which indicates the ability to acquire new genetic material. Another practical aspect of genetic manipulation is genome sequence determination, which Dr. Hildebrand described as a requirement for informed manipulation. The genome sequence enables identification of potential target genes to manipulate, provides a means to derive metabolic maps of the organism, and provides native expression control elements to control and alter the expression of introduced genes. Lastly, tool development is also a critical aspect of genetic manipulation. Regarding diatoms, the focus of Dr. Hildebrand's work, tools developed include selectable gene markers (such as fluorescent tagging), codon optimization, and transformation approaches. The tools developed for one particular diatom species have generally worked in other genera.

In conclusion, Dr. Hildebrand emphasized that genetically engineered organisms are not necessarily genetically modified. Organisms are classified as genetically modified only if deoxyribonucleic acid (DNA) from another genus is introduced. Recombination and crossover can be generated by the native host and are not classified as genetic modification because, in theory, the organism could have done this type of evolution on its own. Dr. Hildebrand identified several priorities for future research, including further refinement of manipulation tools, detailed characterization of carbon flux and metabolism in diverse classes of microalgae, expanded understanding of the factors that control growth rate and biomass accumulation, and application of these approaches to actual production conditions.

Omics Databases

Omics databases are a valuable contribution to the field of algal biology given that they enhance the efficiency of research efforts by sharing previously established research. In this context, omics approaches cover the following:

- **Genomics:** Sequenced genomes are essential for determining the physiological potential of production strains and for strain improvement. Genome size in algae can vary substantially.

- **Transcriptomics:** Analysis of gene expression will illuminate environmental response and enable gene annotation of other species.

- **Proteomics:** Proteomics approaches enable protein identification, quantification and detection of post-translational modifications.

- **Metabolomics and Lipidomics:** The metabolome is the collection of small molecular weight compounds in a cell that are involved in growth, maintenance, and function. Lipids are a subset of the molecular repertoire of the algae cell.

- **Phenomics:** Phenomics will improve understanding of gene functions and environmental effects on cultivation.

Partnerships such as the Joint Genome Institute and the Genome Science Program have begun to establish a baseline for resources and are working to set a standard for the development of future omics databases.

Algal Omics Resources
Shawn Starkenburg, Ph.D.
Los Alamos National Laboratory and New Mexico Consortium – Genome Science Programs

Dr. Starkenburg began his presentation with an overview of the value of genomics data, which includes helping to (1) build fundamental knowledge of algal biology, (2) inform rational genetic engineering (and breeding) strategies, and (3) establish cultivation diagnostics, monitoring, and crop protection methods.

There are several existing genomics databases, such as Phytozome and IMG (Integrated Microbial Genomes), CpBase (Chloroplast Genome Database), MitoBase (Mitochondria Genome Database), MMETSP (Marine Microbial Eukaryote Transcriptome Sequencing Project), NCBI (National Center for Biotechnology Information), GenBank, and greenhouse.lanl.gov. Despite the existence of many databases, there is still much room for improvement in developing comprehensive genomics informational resources. One challenge is the high nucleotide diversity of algae, which makes it difficult to create accurate genomic annotations.

Although it remains challenging to annotate algal genomes, recent developments in long-read sequencing technology now allow for full-length transcripts and nearly complete genome assemblies. Other recent advances in genomics research include scaffolding tools for chromosomal organization, high molecular weight DNA extraction methods, and improved annotation pipelines.

Additionally, there are a few very good analysis packages available for cultivation diagnostics and profiling. Dr. Starkenburg recommended the following tools:

- The Mothur Project: www.mothur.org

- The ARB Project: www.arb-home.de

- Quantitative Insights into Microbial Ecology: www.qiime.org

Dr. Starkenburg also noted that many of the existing ribosomal ribonucleic acid (rRNA) reference databases are biased toward microbes and have very few eukaryotic sequences, which makes them subpar for algae research. Nonetheless, he recommended the following sources as potentially useful:

- Ribosomal Database Project: rdp.cme.msu.edu/classifier/classifier.jsp

- Greengenes: greengenes.lbl.gov/cgi-bin/nph-index.cgi

- Silva: www.arb-silva.de

- NCBI: www.ncbi.nlm.nih.gov/guide/taxonomy/

Bioinformatic analysis platforms, such as CyVerse, KBase (DOE Systems Biology Knowledgebase), Pathway Tools, EDGE bioinformatics (Empowering the Development of Genomics Expertise), Galaxy, Ergatis, and workflow managers, are available to researchers but are limited in scope. Like the rRNA reference databases, there are no workflows and few metabolic models for algae. The existing tools are heavily biased toward bacteria and eukaryotic model systems. Further, the tools are not equipped to handle the complexity of multi-omics experiments due to limited omics integration software and immature statistical measures. Lastly, there are few tools for analyzing time course information and limited or no predictive learning software. According to Dr. Starkenburg, the omics community needs to address these shortfalls by developing a tool that assists with combining multiple existing models.

In closing, Dr. Starkenburg stated that bioinformatics analysis platforms for algae should aim to create the basic knowledge platform that is needed to move into the strain improvement stage. He suggested that an "in silico" resource is needed and that it should enable the functional analysis of all omics data types with the following capabilities:

- Assembly, annotation, and curation tools

- Integration and analysis software (genomics to phenomics as well as push-button workflows)

- Predictive/deep learning applications

- Standardization

- Comprehensive databases/web-based interfaces.

Algal Genomics Toolkit
Igor Grigoriev, Ph.D.
Joint Genome Institute – Fungal Genomics Program

The Joint Genome Institute is a DOE genomics user facility with nearly 1,000 researchers annually utilizing its genome toolkit, which features the following capabilities:

- **Sampling:** Samples compiled in the Joint Genome Institute's Genome Portal, which receives approximately 20,000 visitors per year and offers free full-genome downloads online

- **Sequencing:** Technologies ranging from ultra-high-throughput to long-read equipment

- **Assembly:** Alignment of fragmented genome sequences

- **Annotation:** Automated pipeline that involves gene prediction based off of data in Genome Portal

- **Analysis:** Tools and techniques for algal metabolite analysis that allow for strain comparison and characterization.

In addition to offering researchers access to the genomics user facility, the Joint Genome Institute has annotated more than 20 algal genomes and addressed some of the challenges related to algal genomics, and it continues to work on algal functional genomics in cooperation with Lawrence Berkeley National Laboratory. Dr. Grigoriev stated that new capabilities in metabolomics and molecular tools development, integration of omics data, and metabolic modeling will accelerate the strain improvement process.

Gene Expression and Silencing

In order to achieve focused algal strain improvement, a combination of gene expression and silencing is used to target ideal attributes. Professor Cerutti with the Center for Plant Science Innovation at the University of Nebraska, Lincoln, provided an overview of recent research findings and comments about ongoing efforts.

Silencing of Nuclear Transgenes in Microalgae
Heriberto Cerutti, Ph.D.
University of Nebraska, Lincoln – Center for Plant Science Innovation

Professor Cerutti and his colleagues at the University of Nebraska are working on transgene design, including transcription, ribonucleic acid (RNA) processing and stability, translation, and protein stability and function. Epigenetic gene silencing mechanisms are widespread in algae but have species-specific distribution. Research indicates that newly introduced genes may be suppressed due to gene regulation (related to mating loci or stress responses) or as a defense response against genomic parasites such as transposons. Professor Cerutti noted four key findings: (1) single-copy transgenes with effective promoters are less prone to silencing, (2) single-copy transgenes that escape the initial silencing seem to express stably for multiple generations, (3) post-transcriptional gene silencing is less problematic for transgene expression in *Chlamydomonas*, and (4) techniques have been identified to avoid gene silencing. Continued work remains focused on transcriptional and post-transcriptional gene silencing. These efforts are performed in collaboration with partners such as the National Science Foundation, Nebraska Coalition for Algal Biology and Biotechnology, and Korea Research Institute for Bioscience and Biotechnology.

Co-Products

The December 2015 *Bioproducts to Enable Biofuels Workshop Summary Report* established that the manufacture of algal co-products is a key strategy for achieving commercial-scale algal biofuels. Algal biomass can be used to make a range of valuable bioproducts, such as industrial chemicals, biobased polymers, and proteins. BETO is investing in applied R&D technologies that can achieve higher yields of target bioproducts from algae to increase the overall value for algae biomass. Dr. Lieve Laurens, with the National Bioenergy Center at the National Renewable Energy Laboratory (NREL), discussed recent research developments related to high-value co-products and related production pathways.

An experimental approach to developing algal co-products is similar to agricultural co-production methods. By determining the chemical makeup of postproduction by-products, novel co-products can be discovered. Dr. Stephen Mayfield at the California Center for Algae Biotechnology takes this approach in his product development research efforts.

Co-Products Value Proposition
Lieve Laurens, Ph.D.
National Renewable Energy Laboratory – National Bioenergy Center

Dr. Laurens explained that NREL and its partners in the Algae Biomass Valorization project established two primary goals related to the development of algal co-products. The first goal is reducing the cost of algal biofuels by increasing the inherent value of algal biomass. Current related efforts involve quantifying product costs using a multi-product algal biorefinery model that incorporates the costs of capital, biorefinery operation, and co-products. Work in this area aims to demonstrate and validate rapid, high-throughput compositional analysis technologies, demonstrate dynamic composition and quantitative shortcomings for mass balance accounting, and establish a relationship between conversion characteristics and biomass composition. Another objective is to identify and isolate high-value co-products that are scalable and compatible with novel multi-product biorefinery pathways. Products identified as potentially marketable include the following:

- Epoxies and polyols

- Surfactants and fuel additives

- Biopolymers, coatings, and rubber

- Di-acids for nylon production

- Fermentation products (including ethanol and di-acids).

The second primary goal is reducing uncertainty around major process inputs and outputs in techno-economic analysis. Along with establishing, disseminating, and maintaining analytical tools, methods, and datasets, Dr. Laurens has also worked toward developing the adoption and implementation of technical standards, in partnership with the Algae Biomass Organization. The resulting database of microalgal procedures is available online. NREL hosts this free resource, which provides the algae industry with up-to-date guidance on uniform descriptions for biomass characterization and valorization as well as trading standards for biomass.

Dr. Laurens reported that, as of 2015, techno-economic modeling indicates a biomass production cost of $491 per ton and a co-products biomass value of $470–$600 per ton. In general, the minimum fuel selling price is directly influenced by algal biomass productivity and composition (lipids contribute 40%–72% of the fuel selling price, and carbohydrates contribute 8%–48%). The successful production of algal biomass co-products creates the potential to simultaneously increase biomass value and decrease minimum fuel selling price. Techno-economic analysis can be used to estimate market sizes based on displacement volumes for many key co-products. A next step on this value proposition work will be to determine biomass production scale in order to achieve a saturated market (i.e., calculating how many farms it would take to grow a sufficient level of biomass to meet market demand for a given final output).

Also regarding future toolbox development, Dr. Laurens noted the need to extend existing databases and build new ones related to lipid-based products. She considers this ongoing development necessary to genomics resources, given that metabolomic and lipidomic profiling provide insights into novel co-products. Future work will also need to focus on isolating high-value, large market components and mapping their cultivation and biomass conversion in order to advance the algal bioenergy state of technology.

Photosynthetic Biomanufacturing Co-Products from Green Algae
Stephen Mayfield, Ph.D.
University of California, San Diego – California Center for Algae Biotechnology

Dr. Mayfield began his presentation by defining biomanufacturing as the use of living systems and organisms to produce useful products. He noted that although it may not be common understanding, agriculture is biomanufacturing and "algae is agriculture." Algae contain lipids, carbohydrates, and proteins that can be used to create a wide spectrum of products. Cultivation and harvesting can be performed using open-pond production systems, photobioreactors (PBRs), or fermenters. Each of these systems has advantages and disadvantages, and selection should be determined depending upon the product being made.

The development of novel co-products is an important aspect of improving the economics of algal biofuels, following successful bioprospecting, engineering, and breeding-selection efforts. One potential product studied by Dr. Mayfield and his research team was a bovine colostrum protein. Studies showed protein effectiveness in improvement of symptoms of gastrointestinal disease and reduction of diarrhea. Algal-derived colostrum could serve as a replacement to antibiotics in both human and animal health. The colostrum protein product also contains components that would make it a valuable supplement to baby formula, which has a very large market.

From Flask to Farm

Effective high-volume cultivation and harvest is critical to achieving commercial-scale algal biofuels and bioproducts. Researchers have identified a major challenge in translating laboratory results to large-scale, outdoor environments. National laboratories such as the Los Alamos National Laboratory (LANL) and NREL are working to achieve certainty in taking research results from the laboratory—or the flask—to the farm. Dr. Taraka Dale and Dr. Philip Pienkos both spoke about their efforts to improve predictions about how strains will perform in real-world conditions.

Progress and Challenges in Transitioning Strains to Larger Scales
Taraka Dale, Ph.D.
Los Alamos National Laboratory, Bioscience Division

Dr. Dale began her presentation by explaining that both productivity and consistency challenges must be overcome in order to create a viable algal biofuels industry. She explained that algae strains do not currently grow fast enough or accumulate enough lipids to be economically viable. Since biomass and lipid productivity are the main drivers of cost, strains' productivity must be improved. Further, strains that have shown improved productivity in the laboratory environment often perform differently when grown outside.

LANL's research goal was to develop a streamlined process for improving algae strains and characterizing their performance at multiple scales. The research team developed three lipid-improved algae strains of *Chlorella* and identified one that experienced stable growth and did not crash—or suddenly decline. The targeted strain was named BD4. Interestingly, all of the data for both the parent and sorted strains were similar. Cell sorting did not affect growth in the BD4 strain, which is not always the case for other strains.

The transitioning process followed the steps outlined below:

1. Growth was first simulated with indoor environmental PBRs (Phenometrics) at LANL.

2. Next, growth was tested in climate-simulating indoor ponds at the Pacific Northwest National Laboratory.

3. Finally, growth was tested in outdoor ponds located in Mesa, Arizona, at the Algae Testbed Public-Private Partnership facility (a testbed facility funded by DOE).

Study results found that biomass accumulation over time was different in each of the three settings; however, each system demonstrated a consistent increase in biomass over time. Dr. Dale reported that there is still much to learn about BD4 with respect to carbon partitioning and optimal cultivation conditions. Future research will also include uncovering the molecular mechanisms that are responsible for improved traits in enhanced algal strains.

Lessons in Bioprospecting
Philip T. Pienkos, Ph.D.
National Renewable Energy Laboratory, Bioprocess R&D

Dr. Pienkos presented an overview of a bioprospecting project conducted by the Algae Biotechnology Partnership. The collaboration was developed at the 2013 BETO Algal Biofuels Strategy Workshop, and partners include NREL, Pacific Northwest National Laboratory, and LANL. As Dr. Pienkos reported, the research goal for this project was to identify and develop novel, genetically tractable (easy to genetically engineer), halotolerant (adapted to saline waters) algal strains for deployment in outdoor cultivation.

For this project, NREL revived previously cryogenically frozen algae strains that were collected from natural environments during an earlier bioprospecting effort with the Colorado Center for Biorefining and Biofuels. Partners in that collection effort were the Colorado School of Mines, Colorado State University, and the University of Colorado. Various algae strains were cultivated from water samples around the Southwest and then cryopreserved. The goal was to collect as many genetically diverse specimens of algae as possible.

Once the bioprospected algae strains were revived, seasonal climate conditions were simulated indoors to select candidates for outdoor testing. Genetic toolboxes were developed for top-tier strains, and then two rounds of outdoor testing were conducted in open ponds at the Algae Testbed Public-Private Partnership. Compositional analysis demonstrated that the fastest-growing strains also generated reasonably high lipid content. The resulting strains are being prepared for wide dissemination via licensing arrangements.

In conclusion, Dr. Pienkos explained that modern tools and a focus on desired characteristics provide the basis for successful bioprospecting. It is also clear that genetic diversity is not assured in choosing the most productive

strains to carry forward and that benchmark strains must be run side by side in order to measure superiority. Finally, indoor cultivation systems can be used to predict relative performance outdoors, but establishing a rapid path to outdoor cultivation remains critical.

Biological Improvements in Cultivation

This series of presentations was made by representatives of private sector algae operations and public-private partnerships working to advance algal biology goals through outdoor cultivation of improved strains. Sapphire Energy, Algenol, Global Algae Innovations, and Algenuity are companies that have commercialized algae-based products. The New Mexico Consortium and the Marine Algae Industrialization Consortium are research partnerships that are making important advances in cultivation methods and technologies, funded under BETO's 2015 Target Algal Biofuels and Bioproducts award.

Christopher Yohn, Ph.D.
Sapphire Energy

Sapphire Energy produces algal biofuels for the oil industry as well as algal bioproducts for the nutrition industry. The production process begins with strain development, cultivation, and harvest. Then, the harvested algae continue on to one of two product pathways, either conversion to fuels or formulation for bioproducts. A conversion and extraction technique is used to create scalable drop-in fuels. Alternately, a drying, extraction, and formulation technique is used to create powder and liquid nutritional supplements.

As the company developed its core technology, Sapphire Energy has also created many algal biology tools, including the following:

- Bioprospecting programs
- High-throughput screening of genetically diverse libraries, both genetically modified and non–genetically modified
- Nuclear and chloroplast engineering platforms in multiple algal species
- Protein/metabolite expression platforms
- Protein evolution programs
- An exogenous chloroplast genome
- Systems biology approaches.

Sapphire Energy partnered with the University of California, San Diego, on the first outdoor field trial of genetically engineered algae, which required a consolidated Toxic Substances Control Act Environmental Release Application through the U.S. Environmental Protection Agency. The study focused on ecological risks of genetically engineered algae and found that the effect on biodiversity, species composition, and biomass of native algae was indiscernible from those of wild-type algae. Dr. Yohn noted that consumer products developed from genetically engineered algae may trigger additional regulatory issues and that technical, regulatory, commercial, and consumer elements must be balanced in future efforts.

Dr. Yohn concluded his presentation with a summary of the algal biology toolbox at Sapphire Energy. The company has developed techniques for high-throughput strain screening and has applied strain improvement tools to address key gaps in algal traits and processes. One important lesson learned is that the application of tools requires consideration of strain use—especially regulatory and commercial aspects. Finally, integration across all unit processes is critical to success.

Harlan Miller, Ph.D.
Algenol

Algenol processes algae into two product categories: fuel-grade ethanol and green crude, which is used as gasoline, jet fuel, and diesel. Algenol's proprietary Direct-to-Ethanol® technology was developed through substantial algal biology efforts and PBR advances. The Direct-to-Ethanol® process has four key components: (1) a productive algal strain, which is a proprietary cyanobacteria strain; (2) VIPER™ PBRs, which grow cyanobacteria in saltwater contained in proprietary equipment that is exposed to the sun and is fed carbon dioxide and nutrients; (3) VIPER™ Manufacturing, featuring in-house capability to prototype, manufacture, and deploy PBR equipment at commercial scales; and (4) energy-efficient separation of algal components.

Since low-cost PBRs are key to Direct-to-Ethanol® commercialization, the company has dedicated significant effort to developing advanced equipment. The second generation shifted from an inexpensive flexible film tube to a vertical system with better light-use efficiency and gas exchange dynamics. The third generation of PBR equipment was designed and sized for low-cost commercial applications and features large effective culture volume, fine-scale nutrient delivery potential, improved cleaning efficiency, and plastic formulations for long deployment lifetimes.

Algenol's outdoor cultivation efforts focus on consistency, repeatability, and improved productivity. Dr. Miller emphasized that not only is economics a function of productivity, but productivity is the most significant economic driver. The many factors affecting these outcomes include PBR design and configuration, strain advancement, light-use efficiency, temperature management, carbon dioxide addition, nutrient addition, and ability to clean the PBRs in place.

David Hazlebeck
Global Algae Innovations

Global Algae Innovations has developed advanced algal strains of varieties such as green algae, red algae, diatoms, and cyanobacteria. The company uses carbon dioxide captured from power plant flue gas. Performance metrics include the following:

- 20 cubic meters per hour harvest rate

- 100% harvest efficiency

- No flocculent necessary.

The resulting products after membrane harvest include a crystal clear effluent and a thick paste of more than 18% solids, ash-free dry weight. Mr. Hazlebeck explained that contamination control is an important aspect of the company's commercial operation. The process to keep each pond free of contaminants involves nutrient preloading and cycling, system design with a variety of treatment options, and strain rotation. This methodology has been successful in large-scale operations with only 75 grams of inoculum for 3 tons of algae. The presentation emphasized that while each pond is a monoculture, the overall production system is not.

Global Algae Innovations has also achieved high productivity through engineering improvements. Mr. Hazlebeck noted that while strain improvement is important, there are also cultivation enhancements that affect productivity, and it is critical to develop algal strain attributes under both considerations. In order to create other economical co-products (such as aquaculture or specialty animal feeds), both high-lipid and high-protein content must be achieved.

Henry Taunt, Ph.D.
Algenuity

Dr. Taunt presented about the role of lab-scale PBRs in the algae industry with respect to Algenuity's efforts to cultivate algal biotech. Like other companies, Algenuity has designed a customized bioreactor to address the common challenges with lab-scale cultivation, such as reproducibility of data, ability to accurately model outdoor

conditions, and flexibility of conditions available. Features of the company's proprietary equipment include the following:

- Lighting control

- Temperature control

- Automated in-flask measurements

- pH monitoring and modulation

- Automated gas control.

Algenuity collaborated with researchers at the University of Cambridge, London's Global University, Rothamsted Research, the Scottish Marine Institute, the John Innes Centre, and the Plymouth Marine Laboratory on algae strain improvements and analytics. Efforts focused on modeling diurnal light cycles in various locations with different climatic conditions. Research found that modeling outdoor algae growth requires more than the previously accepted on/off light cycles. Dr. Taunt emphasized that moving algal biotechnology into the synthetic-biology age requires precise lab-scale cultivation. Using the Algem PBR, the company has demonstrated significant reproducibility and the ability to accurately model outdoor conditions.

Richard Sayre, Ph.D.
Los Alamos National Laboratory and New Mexico Consortium

The New Mexico Consortium is an independent nonprofit corporation that was founded as a partnership between LANL, the University of New Mexico, New Mexico State University, and New Mexico Tech. The research group has made progress toward engineering more robust and improved biomass production in algae by focusing on enhanced heat tolerance, biocontainment, and cellobiose heterotrophic boost for algae growth.

Dr. Sayre presented recent research that resulted in the development of a proof of concept for gene switch technology to contain transgenics and to control expression of potentially toxic co-products. The team also demonstrated enhanced biomass and oil yield by feeding cellulosic sugars (nonfood sugars) to commercial algae strains. The efforts to enhance algae strain heat tolerance enabled the closure of ponds for reduced evaporation. Work focusing on improved biomass production resulted in engineered *Chlorella* for enhanced cellobiose metabolism, leading to faster growth. Regarding future research efforts, the group identified a need to develop inbred, sterile lines of diploid algae to stabilize genotypes as well as transgenic traits.

Zackary Johnson, Ph.D.
Duke University and Marine Algae Industrialization Consortium

Professor Johnson leads the Marine Algae Industrialization Consortium (MAGIC), a research team including academic institutions as well as agriculture and energy companies. MAGIC partners include Archer Daniels Midland, Bentley University, Bucknell University, Cornell University, OpenAlgae, Shell Global Solutions Inc., University of Hawaii, University of Nordland, University of Southern Mississippi, and Valicor Renewables. The consortium has worked to combine biofuel and high-value bioproducts to establish a production strategy with the capacity to meet the national Renewable Fuel Standard.

Part one of the research by MAGIC identified algal productivity and co-products as ripe target areas for techno-economic analysis and life-cycle cost analysis. Subsequent efforts established that commercialization pathways including both fuel and feed are most likely to lead to economically feasible commercial-scale algal fuel technology. In order to reach the DOE target of $5 per gallon of biocrude by 2019, algal productivity would need to reach 55 grams per square meter per day. The research team found that introducing high-value co-products such as fishmeal would assist in achieving this ambitious national production rate.

Part two of MAGIC's research focused on increasing algal productivity to a degree that will lead to economically feasible commercial-scale algal fuel technology. Industrial partners provided input to identify marine algae strains. In order to reduce uncertainty in the techno-economic analysis and life-cycle analysis, growth and extraction approaches were used to demonstrate production and efficacy at successively larger scales. Finally, market transformation and commercialization plans were developed in context of the Renewable Fuel Standard.

Research on improved algal productivity found that increased levels of nitrogen had the potential to increase lipid yield by 30 metric tons per hectare per year, an improvement that exceeds the DOE target for 2018. Also, from an agricultural perspective, MAGIC identified basic husbandry steps that can increase productivity. Research focused on potential co-products found marine green microalgae to be a promising source of bioavailable iron. MAGIC research has found that defatted microalgal biomass may provide a unique opportunity to produce healthier, value-added animal products such as seafood, pigs, and chicken with increased omega-3 fatty acids and higher iron content. This stands to benefit the 1.5–2 billion people worldwide who are affected by iron deficiency.

Professor Johnson concluded his presentation by emphasizing that MAGIC is focusing on end-to-end assessment of co-product production, efficacy, and value using multiple, well-defined microalgae feedstocks.

Open Forum Presentations

Workshop attendees were invited to provide 5-minute briefings on topics of their interest, with a suggested focus on achievements to date, current barriers, and outlook for the future of algal biology.

John Benemann, Ph.D.
MicroBio Engineering Inc.

Dr. Benemann's company has established a process for using algae in wastewater treatment and biofuels production. The company has trademarked the approach as the RNEW® Process.

Peter D. Karp, Ph.D.
SRI International

SRI International has developed pathway tools that enable multi-use metabolic databases, including extensive tools for genome informatics, omics data analysis, and pathway informatics.

Pavlo Bohutskyi, Ph.D.
Pacific Northwest National Laboratory

The research team at Pacific Northwest National Laboratory has developed a cyanobacterium-based bioengineering platform using controlled cultivation, engineering, and protein identification tools.

Jianping Yu, Ph.D.
National Renewable Energy Laboratory

Dr. Yu and his research team are working on methods and applications in fluxomics, which have demonstrated cyanobacterial metabolic network plasticity and stimulation of photosynthesis in ethylene-emitting algae strains. The research team has also uncovered a hidden major pathway in central carbon metabolisms that—in contrast to glycolysis—involves no carbon loss.

Paula Sylvia
Unified Port of San Diego

There are opportunities for aquaculture at the Port of San Diego. Suggested algal biofuels companion businesses include mitigation banking, ecotourism, biomedical, and food production. Secondary business lines include fisheries enhancement, restoration, bioremediation, and educational outreach.

Anne Ruffing, Ph.D.
Sandia National Laboratories

The Sandia National Laboratories have expertise in microalgal engineering, covering the areas of metabolic engineering, traditional strain improvement, molecular tool development, and characterization of modified strains. Dr. Ruffing reported that her research group has identified the following challenges and opportunities for algal biofuels:

- Improve productivity through a better understanding of algal metabolism

- Identify a path for elimination of productivity losses from overexpression and adaptive evolution

- Receive institutional approval to grow genetically modified algae, which could benefit the translation of strain properties from the flask to the field

- Further develop the algal toolbox, which is the key to overcoming the remaining barriers.

James W. Golden, Ph.D.
University of California, San Diego

Dr. Golden reported that his research laboratory has developed a collection of over 75 genetic devices for use in diverse strains to accomplish gene knockout, modification, and overexpression.

Michael Connor
Joule Unlimited Technologies Inc.

Joule Unlimited has developed multiple technologies for producing ultra-low carbon fuels, including SolarFT™ Fuels, Helioculture™ Direct Solar Fuels, and hybrid approaches to produce low-carbon diesel, ethanol, jet fuel, and gasoline.

Jürgen Polle, Ph.D.
City University of New York – Brooklyn College

Dr. Polle established the Laboratory for Experimental and Applied Phycology at Brooklyn College, where he is working on developing comparative genomics for the determination of metabolism diversity in green algae. Through this effort, he aims to contribute more high-quality genomes to the field.

Workshop Participant Responses

The purpose of the Algal Biology Toolbox Workshop breakout sessions was to provide a forum to discuss current understandings of algal biology and biochemistry, available molecular toolboxes, omics databases, and other resources to identify challenges and to measure progress toward commercial algal biofuels. The topics were broken into three focus questions covering the challenges of algal biology, ideal algal strain attributes, and resources needed in order to overcome challenges and achieve ideal strains. Participants were asked to first brainstorm responses among their group, then categorize and prioritize the responses.

Three concurrent breakout groups occupied separate rooms and had 10–15 participants per group. Each group was assigned by BETO ahead of the meeting and was composed of participants from a range of disciplines and affiliations. A facilitator led the discussions utilizing ThinkTank software, which is designed to document collective knowledge. Participants brought laptops to contribute input in real time, using ThinkTank as an input collection tool. Following each of the three focus questions, representatives from each group presented results to the plenary.

The results from each of the three breakout groups were reported at various levels of detail, so, to simplify the reporting of each group's responses, this report provides an overview of responses provided by each breakout group. The feedback structure that follows is divided by focus question and then subdivided by breakout group.

Several common themes and top priorities emerged among all breakout groups. Regarding the challenges that must be overcome, themes included achieving high levels of productivity, furthering development of genetic tools and omics approaches, and improving strain selection and screening methods. With respect to ideal strain attributes, top priorities were tolerance to environmental conditions, including temperature, pests, and salt water; ability to effectively harvest for commercial purposes; highly efficient conversion of solar energy; usefulness in a wide range of end products; and lack of toxicity. Lastly, responses to the tools and resources needed focused around advancing basic understanding of the physiology and metabolism of algae, algae screening tools that enable fast and affordable ways to test and characterize each strain's potential performance in large-scale outdoor growing conditions, and certified and accessible algae reference materials and databases.

Focus Question #1: Biology Unknowns

What are challenges in algal biology, specific to genetic tools, that pose significant hurdles to progress over the next 5 years?

Each of the three breakout groups identified priority challenges related to genetic tools by conducting an impact and likelihood analysis on the list of challenges brainstormed by the group. The groups assigned each challenge a score from one to five regarding both the impact of a given challenge on the field of algal biology and the likelihood of that challenge being successfully overcome in the near term. The resulting priorities reflect challenges that have both a significant impact in impeding progress and a high likelihood of being overcome within a 5-year period (given sufficient level of investment).

Breakout Group One

The first breakout group brainstormed a list of 32 challenges ranging from specific informational limitations to broad areas of research needed. Before categorizing the longer list, the group identified the existence of relatively few well-annotated genomes as the most impactful challenge, along with a poor understanding of ploidy and breeding for many algal strains. The challenge ranked most likely to be overcome in the near term was the need for a catalog of genetic manipulation tools for different classes of microalgae, with the goal of targeting which tools need to be developed for what species.
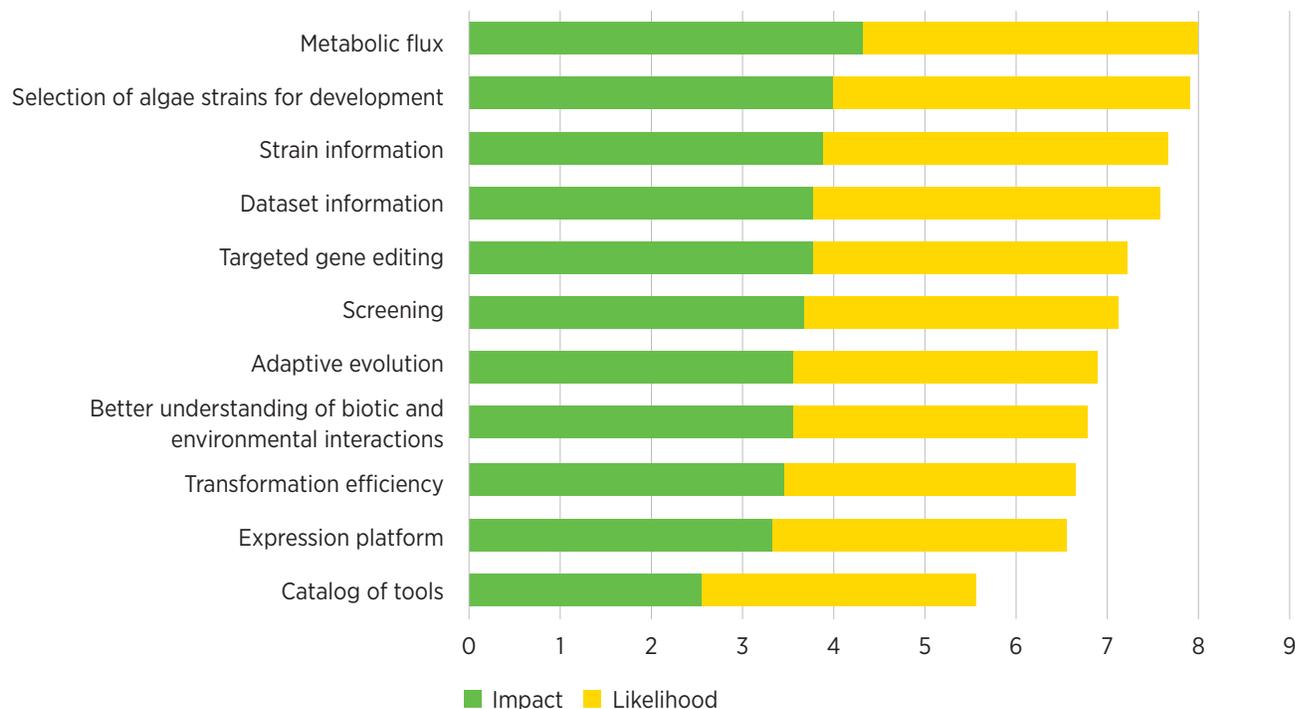
Upon categorizing the list of challenges into common themes, the group arrived at 11 categories. Included in this list was an overarching challenge of coordinating research efforts among various entities to focus extensive

development on common algae organisms that are industrially relevant, rather than many groups studying different organisms and establishing a variety of tools that are not easily adapted for other strains. Broadly categorized, the other challenges were grouped as either information needed (metabolic flux, dataset information and omics databases, and annotation ploidy and breeding information) or tools needed (transformation efficiency, targeted gene editing, screening, and expression platform). The group also noted the need for more data around algae interaction with biotic and environmental factors, as well as a better understanding of adaptive evolution techniques, including tracking phenotype-genotype linkages. Among all of these categories, the first breakout group identified the following priorities:

Table 1. Focus Question #1, Priority Challenges, Breakout Group #1

Rank	Challenge	Impact	Likelihood	Total
1	Metabolic flux	4.33	3.67	8.00
2	Selection of algae strains for development by all groups developing algal biology tools	4.00	3.89	7.89
3	Strain information	3.89	3.78	7.67
4	Dataset information	3.78	3.78	7.56
5	Targeted gene editing	3.78	3.44	7.22
6	Screening	3.67	3.44	7.11
7	Adaptive evolution	3.56	3.33	6.89
8	Better understanding of interaction with biotic and environmental factors	3.56	3.22	6.78
9	Transformation efficiency	3.44	3.22	6.67
10	Expression platform	3.33	3.22	6.56
11	Catalog of tools	2.56	3.00	5.56

Figure 3. Focus question #1, priority challenges, breakout group #1

Breakout Group Two

The second breakout group brainstormed a list of 28 challenges in algal biology ranging from broad, fundamental understanding of industrial algae strains' basic biology to methods for crop protection. These challenges were categorized into seven themes, including relevant species, genome availability, understanding of wider pond communities, molecular biology tools, bioinformatics tools, scaling, and systems biology.

This group determined that the following were priority challenges in algal biology to be addressed by research in the near term:

- Methods to select strains that can be mass cultured as a starting point for algal mass culture and genetics platform

- Understanding of how algae communicate and are affected by microbes surrounding the ponds in which they grow

- Tools and methods for crop protection against contamination and predatory species

- Automated genome annotation and curation that links to omics data and is available to engineers

- Data sharing platform for omics sequencing projects

- Understanding of whether improved strains will be stable over time despite factors such as genetic drift and epigenetic regulation

- Availability of and access to annotated genomes for a subset of organisms representing major groups of deployable organisms

- Robust and reliable tools for gene expression in production strains

- Genome sequencing

- Development of extraction methods to purify high molecular-weight DNA to take advantage of next-generation sequencing platforms.

One participant provided a summary of the genetic tools generally utilized in algal biology. Those include transformation efficiency, genome editing, annotation, stability of genotype/phenotypes, and genome sequencing. This group member commented that there are ample opportunities to conduct long-term testing in relevant outdoor scenarios. Another participant noted that *Spirulina* is a good example of an organism that can be mass cultured with little or no genetics work.

A full list of priority challenges and a summarized ranking of priority challenges from this breakout group are listed below in Table 2 and Figure 4, respectively.
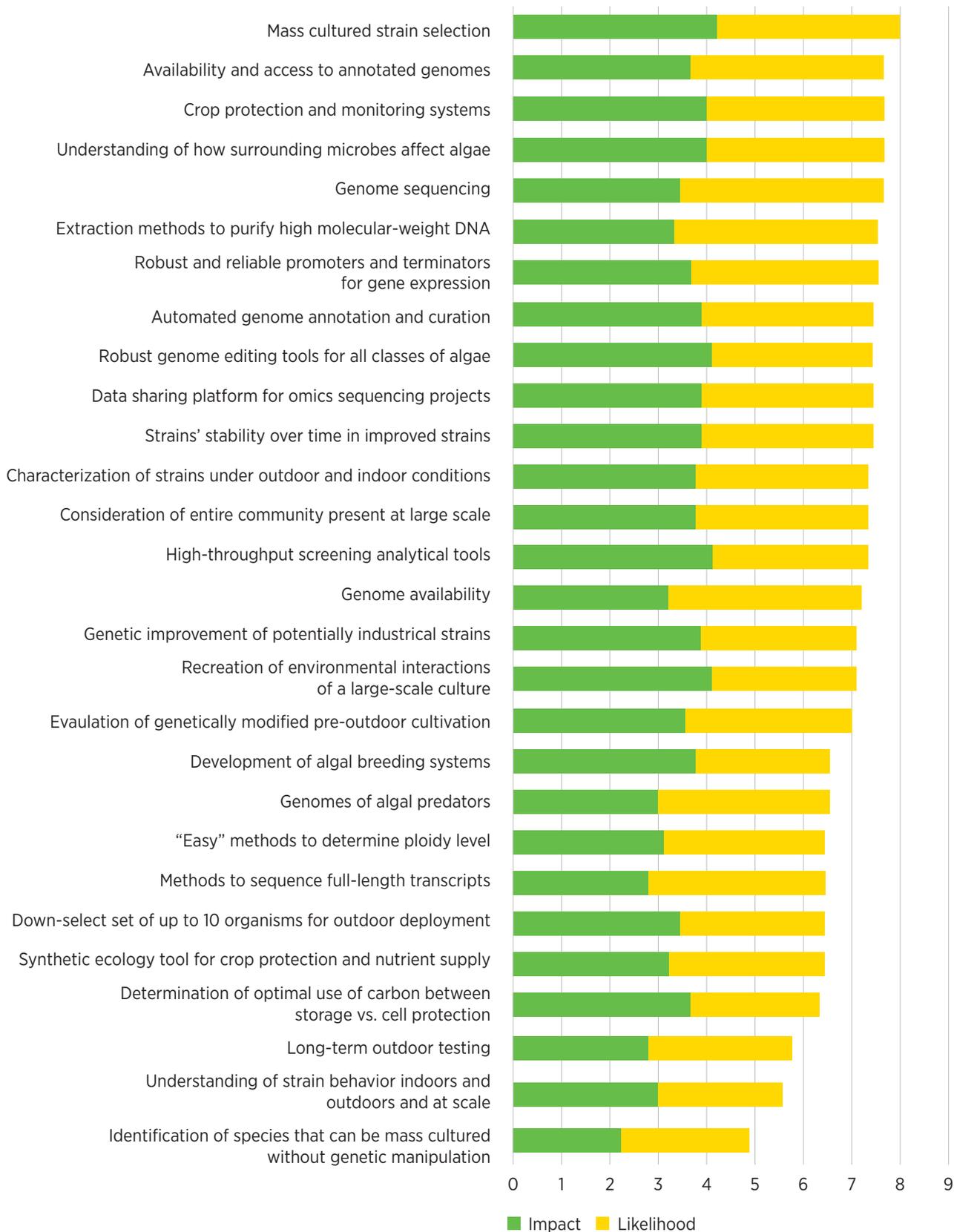
Table 2. Focus Question #1, Priority Challenges, Breakout Group #2

Rank	Challenge	Impact	Likelihood	Total
1	Methods to select strains that can be actually mass cultured, as a starting point for algal mass culture and genetics platform	4.22	3.78	8.00
2	Availability and access to annotated genomes for a subset of organisms representing major groups of "deployable" organisms	3.67	4.00	7.67
3	Tools and methods for crop protection against contamination and predatory species monitoring	4.00	3.67	7.67
4	Understanding of how algae communicate and are affected by microbes surrounding algae (algae do not grow and function as axenic cultures)	4.00	3.67	7.67
5	Genome sequencing	3.44	4.22	7.67
6	Development of extraction methods to purify high molecular-weight DNA to take advantage of modern next-generation sequencing platforms	3.33	4.22	7.56
7	Robust and reliable "tool parts" (promoters, terminators, etc.) for gene expression in production strains	3.67	3.89	7.56
8	Automated genome annotation and curation, and linking to omics data—make these techniques available for engineers	3.89	3.56	7.44
9	Robust genome editing tools for all classes of algae	4.11	3.33	7.44
10	Data sharing platform for omics sequencing projects	3.89	3.56	7.44
11	Understanding if improved strains will be stable over time (genetic drift, epigenetic regulation, genotype vs. phenotype)	3.89	3.56	7.44
12	Characterization of stains under varying conditions—outdoor and indoor	3.78	3.56	7.33
13	Consideration of entire community present at large scale (not just primary algae present)	3.78	3.56	7.33

Table 2 *(continued)*

Rank	Challenge	Impact	Likelihood	Total
14	Appropriate high-throughput screening analytical tools that can select not only for traits of interest (high lipid), but also for the ability to grow to high productivity outdoors at larger and larger scales, which is not only a function of light and temperature or composition of outdoor community in algae pond	4.11	3.22	7.33
15	Genome availability	3.22	4.00	7.22
16	The need for a more fundamental understanding of the basic biology of potential industrial strains, which is required to inform the genetic improvement of these lines	3.89	3.22	7.11
17	Inability to recreate the vast number of environmental interactions of a large-scale culture at an intermediate scale	4.11	3.00	7.11
18	A "scale down" approach to outdoor cultivation for commercial relevance with appropriate parameters to allow evaluation of genetically modified or selected strains	3.56	3.44	7.00
19	Development of algal breeding systems (taking advantage of recombination, hybrid vigor, etc. for algal improvement in similar ways to crop plants)	3.78	2.78	6.56
20	Genomes of algal predators	3.00	3.56	6.56
21	"Easy" methods to determine ploidy level	3.11	3.33	6.44
22	Methods to sequence full-length transcripts to improve annotation	2.78	3.67	6.44
23	Down-select set of up to 10 organisms that represent biological/biochemical/metabolic diversity to build on for different applications of outdoor deployment	3.44	3.00	6.44
24	Synthetic ecology tool to apply for crop protection, for supply of nutrients (e.g., nitrogen) and vitamins, for protection from environmental stresses, etc.	3.22	3.22	6.44
25	Biological tradeoff of carbon use in the algae for storage vs. molecules to protect the cell	3.67	2.67	6.33
26	Chances and opportunities to do long-term testing in relevant outdoor scenarios. What do we consider genetic tools? Transformation efficiency, genome editing, stability of genotype/phenotype, genome sequencing, annotation.	2.78	3.00	5.78
27	Algae strain behavior that changes drastically when cultivated indoors vs. outdoors and with additional scale-up after being brought outdoors	3.00	2.56	5.56
28	Organisms, like Spirulina, that can be mass cultured with little or no genetics work on them	2.22	2.67	4.89

Figure 4. Focus question #1, priority challenges, breakout group #2

Breakout Group Three

The third breakout group brainstormed a list of 29 challenges in algal biology, including increasing the speed to take strains from the lab to a testbed and narrowing down the number of algae strains with which tools are being developed. The full list was organized into seven categories related to breeding, regulation, scaling, microbial ecology, crop protection, omics, and genetic tool development.

As an example of a breeding challenge, one participant noted that there is currently difficulty in optimizing multiple properties simultaneously. Multiple group members mentioned challenges related to the genetic manipulation of algae and the need for clarity around future regulatory acceptance. Challenges involving scaling primarily consist of the translation of laboratory results to algae testbeds. Regarding microbial ecology, participants noted the need to consider the "microbiome" at the farm scale, because the outdoor environment is more complex than flask-scale monocultures. With respect to omics, participants commented on the need for more high-quality genetic sequences that include full annotation for potential production strains. Lastly, in the genetic tool development category, group members discussed the need for genetic markers to follow offspring and explained that there is also a need for counter selection tools for marker recycling and marking removal. Further, they noted that genetic stability was an area in need of improved understanding.
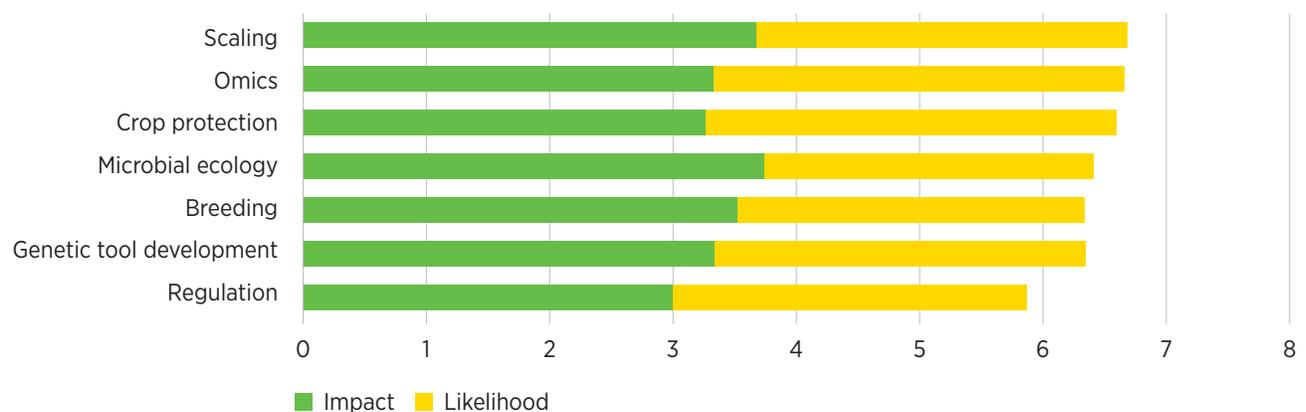
Among all of these challenges, the third breakout group identified scaling, omics, and crop protection to be their top-priority near-term challenges within algal biology.

A ranking and a full list of prioritized algal biology challenges are below in Table 3 and Figure 5.

Table 3. Focus Question #1, Priority Challenges, Breakout Group #3

Rank	Challenge	Impact	Likelihood	Total
1	Scaling	3.67	3.00	6.67
2	Omics	3.33	3.33	6.67
3	Crop protection	3.27	3.33	6.60
4	Microbial ecology	3.73	2.67	6.40
5	Breeding	3.53	2.80	6.33
6	Genetic tool development	3.33	3.00	6.33
7	Regulation	3.00	2.87	5.87

Figure 5. Focus question #1, priority challenges, breakout group #3

Focus Question #2: Species of Focus

Part One: What does success look like; what attributes would the ideal commercial strain(s) possess? What traits should we target for engineering?
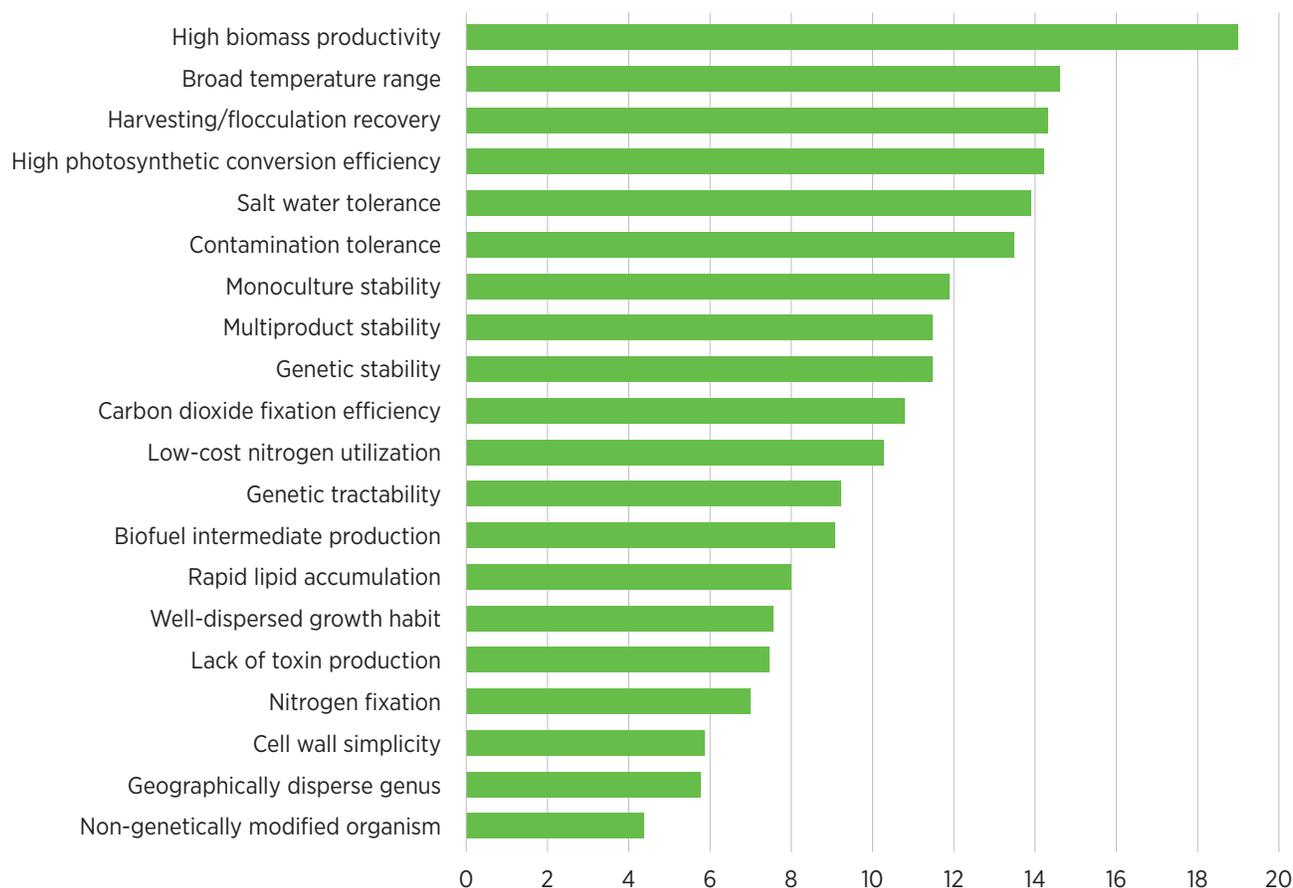
Part Two: How have existing production strains performed against these attributes?

Each of the three breakout groups discussed what success means in relation to strain attributes. Participants also articulated current strain benchmarks and related key metrics needed to achieve success. First, each group brainstormed the ideal strain attributes, then categorized and ranked the attributes according to their importance to commercial production.

Breakout Group One

The first breakout group ranked biomass productivity as the most important commercial strain attribute. One participant noted that in addition to contributing to a higher harvest yield, sufficiently high biomass productivity had the added benefit of minimizing the outgrowth of competing algal species. This participant also noted that algal growth (and thus, productivity) is dependent upon carbon fixation processes, most commonly in the form of photosynthesis. Also related to productivity are strain attributes such as high photosynthetic energy conversion efficiency, light penetration, ability to grow in a stable monoculture, and the consistency of these metrics at larger scales. With respect to "scalability," it was also noted that research has found that large-scale ponds have approximately 80% of the productivity generated in laboratory settings. A full list of strain attributes ranked by priority is below in Figure 6.

Figure 6. Focus question #2, priority strain attributes, breakout group #1

Low-cost production traits were an important category of strain attributes identified by this group. Participants noted that the algae crop must be easily harvested using low-cost technologies, which can be measured using kilowatt-hours (of electricity produced) per kilogram of algae biomass harvested. A general rule of thumb was also suggested: Harvesting operating expenses, including the cost of capital, should be less than 10% of the total production cost. Another factor that contributes to low-cost production is the ability to use inexpensive agricultural grade nitrogen. Participants noted that efficient nitrogen fixation was an ideal trait, although they also explained that research has found no significant loss in productivity when algae is grown in urea or ammonia compared with nitrate.

The group also discussed pH levels and the alkalinity dependent effect on algae. Algae were noted to grow efficiently at a pH greater than or equal to 8 and an alkalinity of 2 milliequivalents per liter. Regarding the production phase, participants explained that biofuel intermediates should be produced along with other co-products that have been identified as having a large market and high market value. It is also important that the products be easily recovered from dilute solution, which is aided when a strain's cell wall composition lends itself to relatively simple metabolite extraction. This extraction rate can be measured in milligrams per liter per hour or in grams per liter per day, depending on the scale of cultivation.

This group also discussed factors that influence a given strain's ability to survive in certain environmental conditions. The group identified ideal strain attributes, such as the ability to grow in a broad temperature range and resistance to viruses and predatory bacteria. Participants suggested a goal of 70% productivity measured in grams per square meter per day as a maximum between annual temperature extremes. The group also noted that some algal strains undergo a shift in biochemical makeup when there is variability in environmental conditions, such as temperature change. The ideal algae strain will have a consistent biochemical makeup despite cultivation conditions. This group also preferred self-suspended systems compared to attached-growth systems, which tend to be more likely to form biofilm.

The genetic system of an algal strain is another category of ideal strain attributes identified by this group. A strain should be genetically tractable and easily to manipulate, while remaining stable. The group noted that if a strain is amenable to manipulation by molecular tools, then it can be transformed genetically. This tractability can be measured in colony-forming units per million cells. A participant also remarked that efficiency is less important when the quantity of cells being transformed can be increased. Another participant commented that it is preferable for commercial purposes if an algal strain can be grown outdoors through many cycles without any genetic drift. Strain stability through manipulation can be measured by productivity metrics, such as grams per square meter per day. A stable strain will have about 95% of the initial productivity after 1 year of unselected growth.
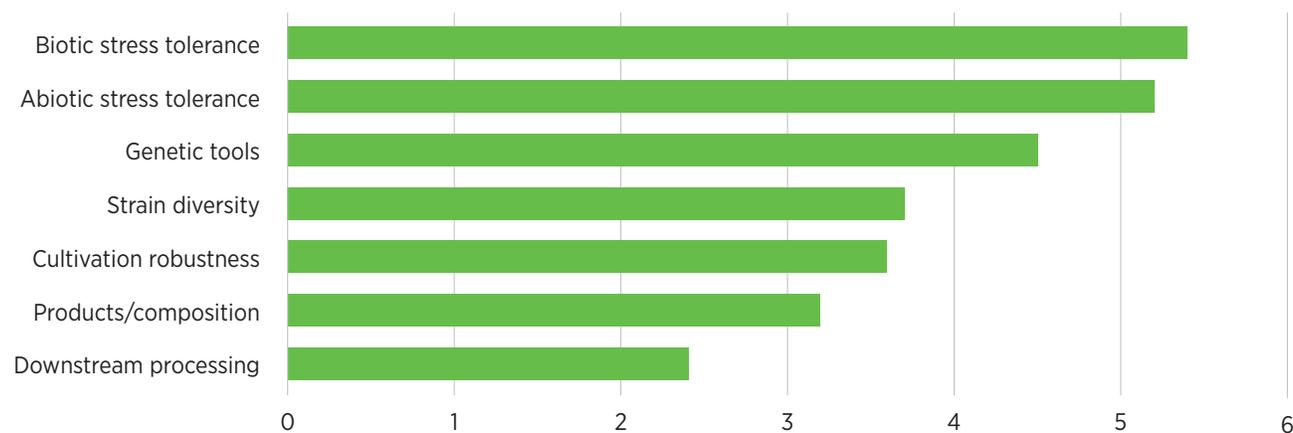
Finally, the group discussed ideal strain attributes related to regulatory and public relations considerations. Participants noted that many communities are more likely to support production using strains that are found local to their region; thus, strains from a genus that can be found naturally in multiple global locations are ideal. One group member suggested that in bioprospecting efforts, information should be collected about the geographic distribution of strains across the globe. A contrasting opinion was also voiced, saying that species from different places may be more productive than some local strains, so this approach may not always be possible.

Another factor that influences public acceptance of algal bioproducts is safety, for example, whether a strain produces a toxin. The group also discussed a favorability for strains that are saltwater tolerant. The presence of this trait allows for cultivation without using increasingly valuable freshwater resources. A proposed metric to determine success with respect to saline tolerance is algal growth in water with salt composition between 5 and 45 parts per ton.

Breakout Group Two

The second breakout group identified ideal strain attributes that echoed many of the traits outlined by the first breakout group, although the traits were categorized differently. The participants in breakout group two voted that biotic and abiotic stress tolerance were the most important strain improvement goals in order to achieve success in commercial algae production. A full list of ranked priority strain attributes is shown below in Figure 7.

Figure 7. Focus question #2, priority strain attributes, breakout group #2

The first factor identified as affecting biotic stress tolerance was grazer deterrence within the cultivation system, which the group noted was a primary requirement for any production system. A group member noted that several strains may be needed to resist multiple different predators, grazers, and infections that could affect a crop of algae. Robustness of culture despite predators and competitors was reported as the second factor related to biotic stress tolerance. Participants recommended that this "robustness" be measured by algal productivity, crash frequency, and the presence or absence of a microbiome. A follow-up comment indicated that green farming tools are available that allow algae strains to naturally coexist with predators or pathogens.

The group identified ideal strain attributes related to abiotic stress tolerance, such as a wide range of temperature tolerance, flue gas tolerance (which one participant noted was not a major issue), growth under variable salinity, and tolerance to high salinity and pH levels.

The participants also identified responsiveness to genetic tools as a category of algae strain traits that is important to commercial productivity in the near term. Ideal attributes include a genetically tractable system, which can be indicated by transformation efficiency with genetic stability and protein expression. Metabolic plasticity is another ideal trait. Lastly, participants noted that, preferably, an *in silico* metabolic model as well as a biology toolkit should be available.

Cultivation robustness—including high productivity, low energy input requirements, and mechanisms for sensing and rapidly adapting to stressors—was ranked as an ideal strain attribute as well. The group suggested that productivity be measured as an annual average. One participant noted that the energy input required in cultivation is a fundamental engineering parameter.

The products and composition attribute category included factors related to marketable co-products measured on the basis of dry weight and desired biochemical composition. Biochemically, the ideal commercial strain will need to have a high carbohydrate or lipid content. Standardized methods of measurement will assist with assessing success related to these factors. Participants recommended dynamic monitoring of biomass composition integrated with production. The group suggested metrics such as the energetic content of biomass as determined by a carbon/hydrogen/nitrogen elemental analysis and the percentage dry weight composition of biomass on an ash-free basis.

This breakout group also identified a need to minimize strain diversity. The participants suggested identifying a subset of 10 optimal strains selected based upon prioritized lists of metrics identified for deployment in 5–10 target locations. Those metrics could include annualized productivity at testbed scale in 5–10 climatically different locations, the ability to grow and collect biochemical composition over at least eight seasons, and a unique catalogue identification of strains based on genetic information.

Another category identified by this breakout group was the ability to perform well in downstream logistics and processing. One ideal strain attribute related to this category is ease of harvest, which can be measured by flocculation response to a standardized test, the purity of return water, or the percent efficiency of the harvest. The group noted that cell wall composition was an attribute complimentary to downstream processes; cell wall composition can be measured by the energy from the algae crop, the cost to extract products, or, more broadly, the product recovery. A final related attribute is the ability to induce processing, as measured by a demonstrable decrease in energy input for downstream processing.

Breakout Group Three

Like the first breakout group, the third breakout group also identified high productivity as the most important algal strain attribute for commercial development, grouping this attribute under the category of cultivation. A full list of the priority strain attributes is shown in Figure 8. The priority strain attribute categories identified by this group include composition, crop protection/stability, cultivation, separations, and strain development.

The cultivation category also included the attributes high biomass yield and broad environmental tolerance, as measured by a percentage decrease in yield/productivity as a function of environment. Tolerance to a wide range of water chemistries was also identified as a relevant cultivation trait, defined by either grams of biomass per square meter, range of parts per million of salt tolerance, or range of pH tolerance. Participants noted that high solar energy conversion efficiency is also necessary, using the metrics of (1) photons per area per time, (2) energy content of fixed biomass compared to solar radiation, or (3) quantum yield indicated by moles of carbon compared to moles of photons. The group also included high temperature tolerance, as measured by rates of productivity compared to temperature, as well as low temperature productivity, using a metric of grams of ash-free dry weight per square meter per day compared to temperature. Efficient nutrient utilization (as measured by nitrogen, phosphorous, and potassium ratios in harvested water) and oxygen tolerance (as measured by the percentage decrease in yield per productivity as a function of percentage of oxygen saturation) were additional ideal cultivation traits, as was photosaturation level, which is defined either by carbon uptake compared to irradiance or by oxygen evolution as a function of irradiance.

The breakout group identified the following commercial strain attributes related to composition:

- Constitutive lipid level, as measured by either omega-3 content, lipid content during nutrient-replete growth, or percentage of fatty acid methyl ester

- Lipid accumulation rate, as measured by grams of fatty acid methyl ester per liter, or per square meters, per day

- Level of all essential amino acids, as a percentage of total protein

- The bioaccumulation of precious metals, measured by carat weight at harvest or atomic absorption spectroscopy.
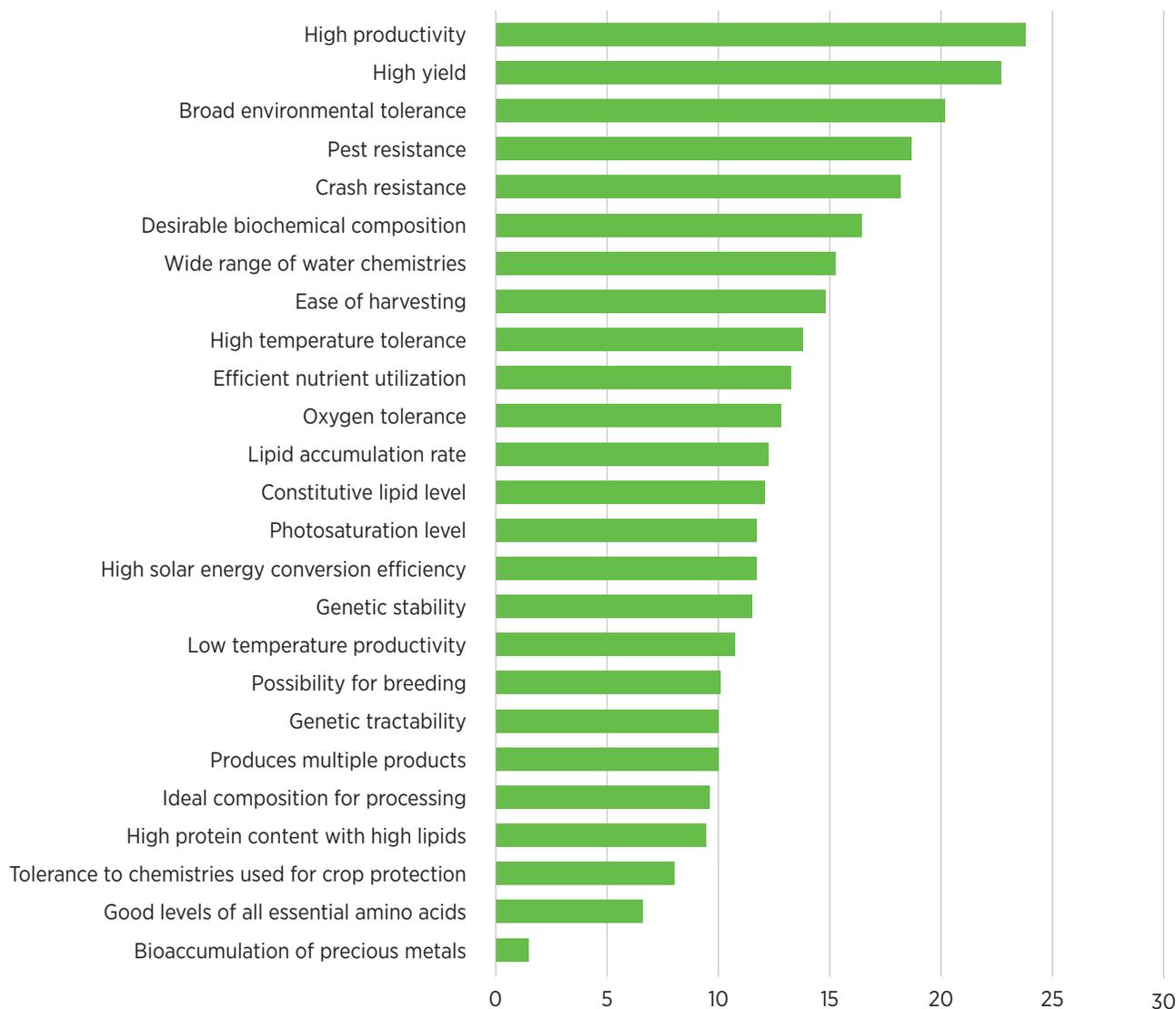
Discussing the attribute of high lipid levels, participants commented that there is a need to develop a relationship between composition and yield, or quantity of hydrothermal liquefaction oil, as well as a need to distinguish between triacylglycerol, free fatty acids, and polar glycerolipids. In discussing metrics to identify algae strains with high protein content, one participant commented that protein is difficult to quantify. Other group members

suggested using absorbance at a wavelength of 280 nanometers to provide an estimate of protein content if extracted. Another group member recommended the Bradford spectrophotometric assay method to measure protein.

According to the group, a desirable biochemical composition involves the factors of amino acid profile, ash content, and pigments. Participants also noted the fact that some production processes have different ideal algal biochemical compositions, and algae strains should be developed accordingly. In order for an algal strain to be beneficial for the production of a variety of co-products, the group members suggested that important factors were omega-3 content, pigment content, and carotenoid content.

Another category of ideal strain attributes identified by this breakout group was crop protection and stability, also defined as robustness, which included traits such as pest resistance and crash resistance. Also discussed was the importance of strain attributes in the separations category, such as the ease of lipid extraction and harvesting. Ease of harvesting can be measured by the energy required, settling rate, filtration rate, foaming from extracellular material, and quantity of extracellular material. Ease of lipid extraction can be indicated by the percentage yield in extract, the percentage of fatty acid methyl ester purity of lipid extract, and the solvent extraction efficiency without the performance of cell lysis (which effectively breaks open the cell membrane).

Figure 8. Focus question #2, priority strain attributes, breakout group #3

Focus Question #3: Future Directions

Part One: What tools and resources do we have at our disposal to overcome the challenges of algal biology and to achieve ideal strains?
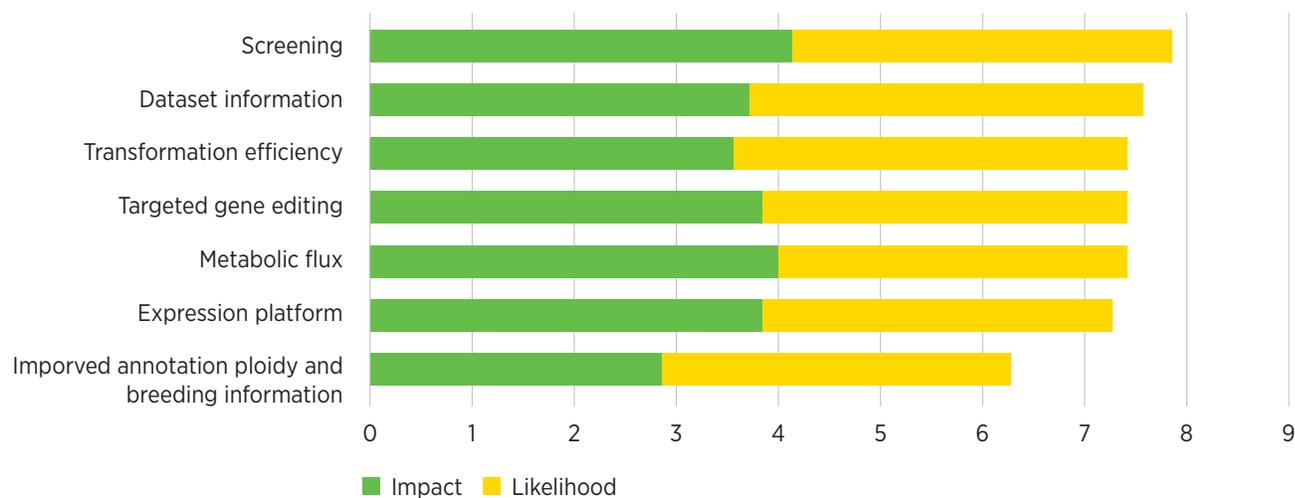
Part Two: What tools and resources are still needed?

Each of the three breakout groups examined the input provided in response to focus questions 1 and 2. Discussion focused on implementation plans to achieve the goals identified in previous focus questions. A few of the specific points covered by participants included: What does successfully overcoming algal biology challenges look like? What are successful models for genetic tool development from other fields, and can they be applied to algae? Breakout groups were also asked to consider whether further bioprospecting efforts may be needed and whether genetic engineering is necessary to meet current algae strain development goals. By assigning scores from one to five regarding both the impact of a given tool or resource on the field of algal biology and the likelihood of that resource being successfully developed in the near term, each of the groups prioritized the most important resources to focus investment and research efforts.

Breakout Group One

The first breakout group populated their initial list of tools and resources still needed with a set of challenges that they had brainstormed during the first focus question, which was categorized as "tools and information." The informational resources identified as currently incomplete were metabolic flux, dataset information, and improved annotation ploidy and breeding information. The tools identified as currently underdeveloped were transformation efficiency, targeted gene editing, screening, and expression platform. A full list of priority tools and resources still needed is below in Figure 9.

Figure 9. Focus question #3, priority resources needed, breakout group #1

Discussion around the top priority category, screening tools, included three points of guidance from participants. One group member suggested that improved screening tools could aid the utilization of adaptive evolution techniques. Another participant described the ideal screening tool as one which enables detection of microbial contaminants in a mass cultivation setting, while also being affordable and fast. A final group member defined the ideal screening tool as having the ability to screen many different strains in a high-throughput fashion under outdoor, relevant conditions.

Conversation about the dataset information category included comments that only very limited genomics information is currently available for algae. Researchers would benefit from having richly populated databases that described genomes and metabolism of key algae strains. Also, a data tool that combined sequencing of a broad and diverse set of algae with deep omics would improve the annotation of selected models. In defining the omics datasets needed for selected strains, participants mentioned several different categories including transcriptomics, proteomics, lipid and carbohydrate profiling, and epigenomics (datasets that are ENCODE-like [Encyclopedia of DNA Elements], encodeproject.org).

Breakout Group Two

The second breakout group discussed a broad set of tools and resources needed for the advancement of algal biology in the near term. The three items below all received a top impact score:

- Advance basic understanding of algal physiology and metabolism (particularly for a few diverse species with production potential)

- Develop robust molecular/genetic toolbox for key chlorophytes (including genome editing techniques)

- Determine the ideal scale/system size/volume that is predictive of outdoor cultivation and provide access to a mid-throughput screening platform to bridge the lab-to-field gap.

The group decided that the need for advancing scientific understanding of algal physiology and metabolism was the most likely resource to be achievable in the next 5 years, making it the top near-term research priority for this group. Also of note was the recommendation to focus this work in physiology and metabolism on a few select algae species with high production potential. The second resource that received a top average impact score was the development of a robust molecular and genetic toolbox for key chlorophytes. This resource was ranked as the second top research priority for this breakout group. The group voted that determining the ideal laboratory algal volume to predict outdoor cultivation effectiveness in the field was slightly less likely to be achieved in the near term.

The group predicted that the algal biology field would be fairly likely to develop automated annotation tools for novel genomes as well as reference databases for species identification. Participants ranked the development of a centralized repository for all omics profiles as another important effort in the field. This central depository could benefit from another priority the group identified: the standardization of multi-omics characterizations for each production strain using a defined set of experiments. Another related resource suggestion from the group was basing lipidome and metabolome databases on mass spectrometry in order to assist with identifying novel products and to support the discovery of novel components. This could add value given that existing databases are mostly developed for human and microbial lipids and metabolites, while plant and algae databases are not well documented in an open format. Figure 10, below, illustrates the abbreviated rankings of priority tools and resources identified by this group. The full list of tools and resources still needed is shown in Table 4.

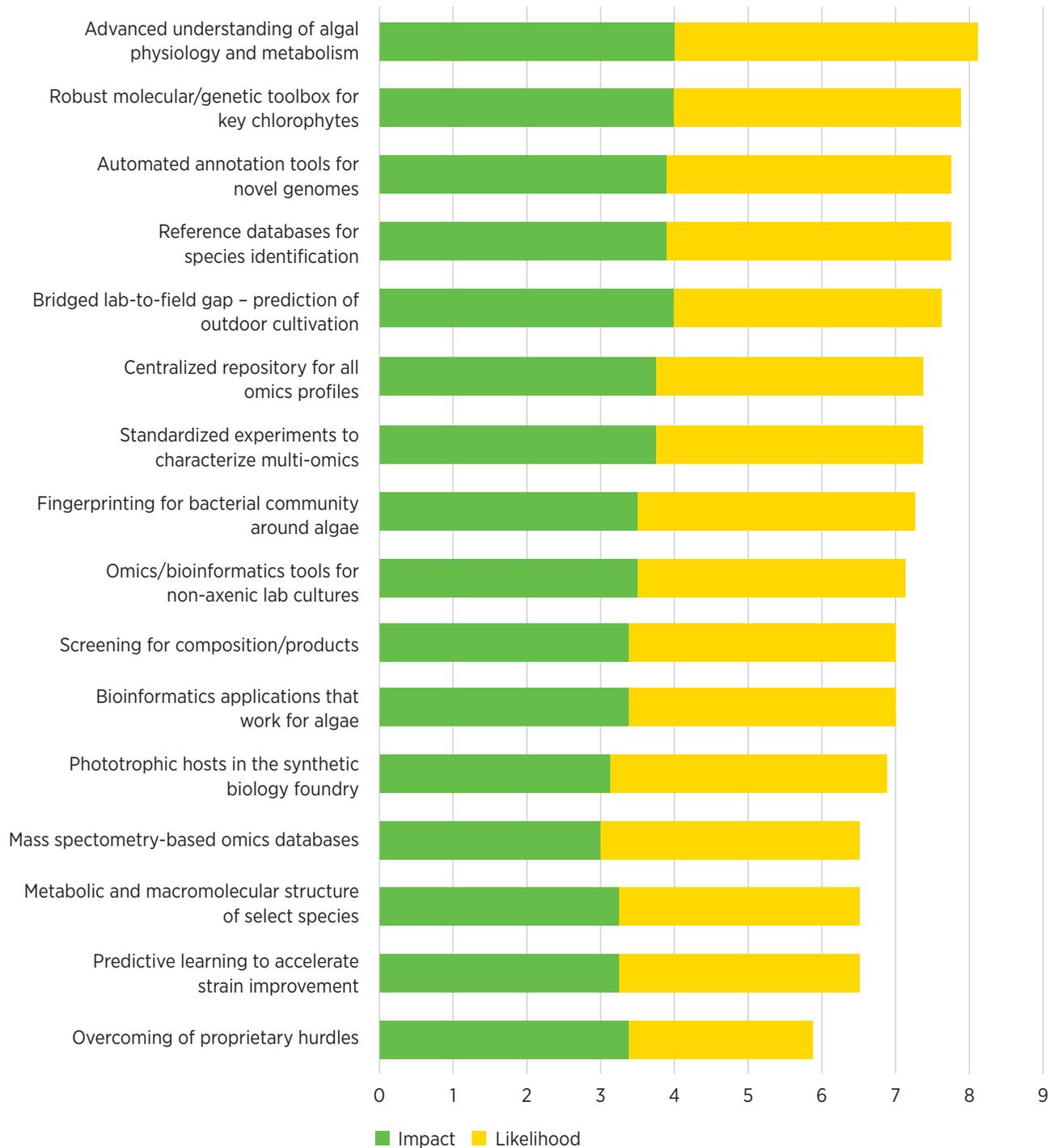**Figure 10. Focus question #3, tools and resources still needed, breakout group #2**

Table 4. Focus Question #3, Tools and Resources Still Needed, Breakout Group #2

Rank	Tool/Resource	Impact	Likelihood	Total
1	Advanced understanding of algal physiology and metabolism (particularly for a few diverse species with production potential)	4.00	4.12	8.12
2	Robust molecular/genetic toolbox for key chlorophytes (including genome editing techniques)	4.00	3.88	7.88
3	Automated annotation tools for novel genomes	3.88	3.88	7.75
4	Reference databases for species identification	3.88	3.88	7.75
5	Determination of ideal scale/system size/volume that is predictive of outdoor cultivation, access to a mid-throughput screening platform, and bridging of the lab-to-field gap	4.00	3.62	7.62
6	Centralized repository for all omics profiles (genomics through phenomics)	3.75	3.62	7.38
7	A defined set of experiments to conduct multi-omics characterization of each production strain (standardization)	3.75	3.62	7.38
8	Fingerprinting tools for bacterial community around algae	3.50	3.75	7.25
9	Omics and bioinformatics tools for non-axenic cultures of algae/cyanobacteria	3.50	3.62	7.12
10	High-throughput quantitative, validated screens for primary composition and presence of co-products	3.38	3.62	7.00
11	Bioinformatics applications that work for algae	3.38	3.62	7.00
12	Phototrophic hosts in the "synthetic biology foundry"	3.12	3.75	6.88
13	Mass spectrometry-based databases of lipidome and metabolome, identifying novel products	3.00	3.50	6.50
14	Understanding, documentation, and dissemination of the metabolic and macromolecular structure of a select set of species integrated with controlled cultivation conditions	3.25	3.25	6.50
15	Application and development of predictive learning methods to accelerate strain improvement	3.25	3.25	6.50
16	Overcoming of proprietary hurdles while still generating information on strains that are commercially useful	3.38	2.50	5.88

Breakout Group Three

The third breakout group arrived at five categories of resources that needed further development within the algal biology field, including omics tools, reference tools, cultivation tools, genetics tools, and sheer luck. Ranked specific resources are illustrated in Figure 11, and a full list is below in Table 5.

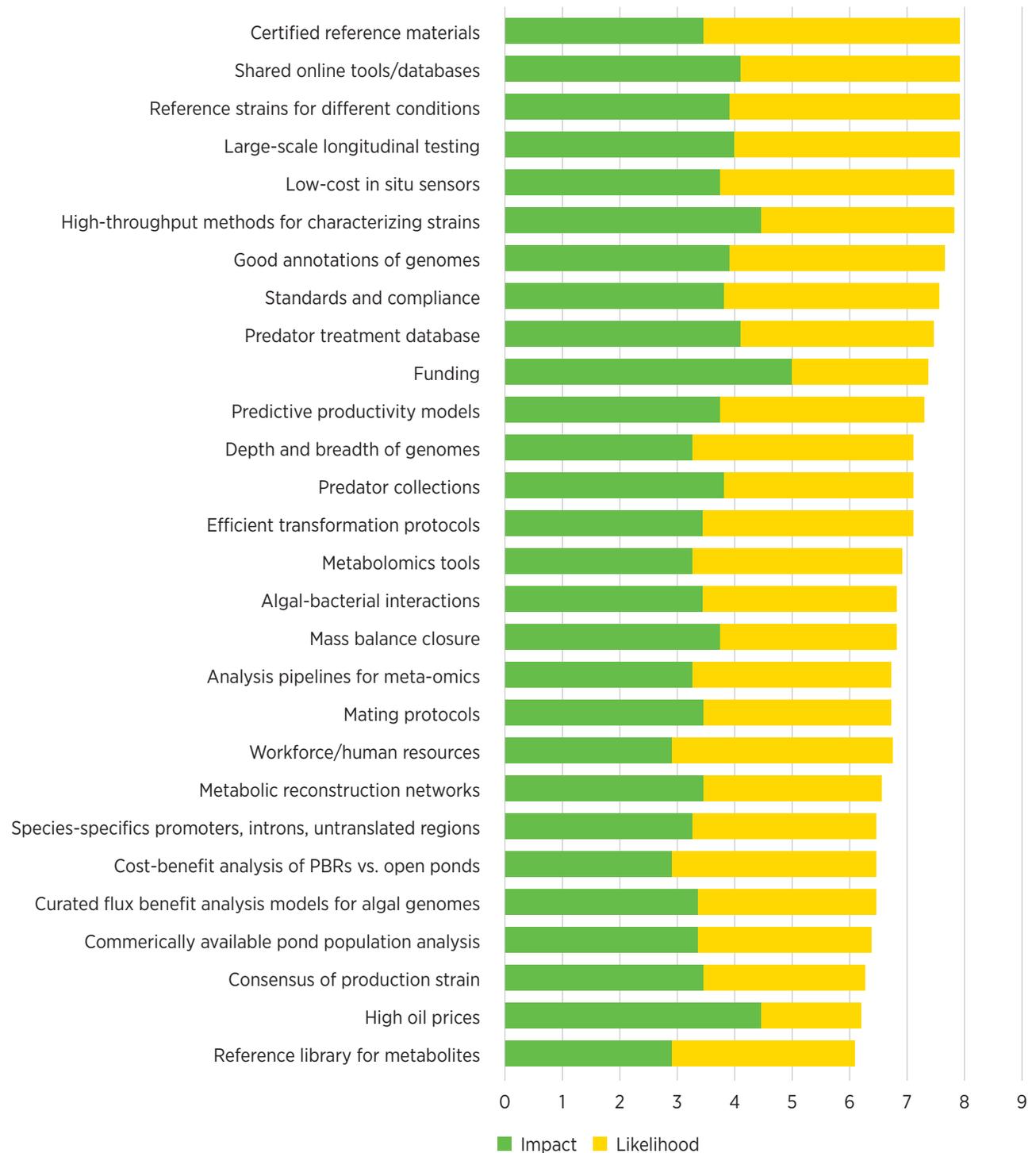**Figure 11. Focus question #3, tools and resources still needed, breakout group #3**

Table 5. Focus Question #3, Tools and Resources Still Needed, Breakout Group #3

Rank	Tool/Resource	Impact	Likelihood	Total
1	Certified reference materials	3.45	4.45	7.91
2	Online tools/databases for community sharing of data and resources on algae	4.09	3.82	7.91
3	Reference strains for different conditions (high temperature, low temperature, etc.)	3.91	4.00	7.91
4	Large-scale longitudinal testing	4.00	3.91	7.91
5	Low-cost *in situ* sensors	3.73	4.09	7.82
6	High-throughput methods for mimicking outdoor conditions and characterizing strains	4.45	3.36	7.82
7	Good annotations of genomes	3.91	3.73	7.64
8	Standards and compliance	3.82	3.73	7.55
9	Predator treatment database	4.09	3.36	7.45
10	Funding	5.00	2.36	7.36
11	Predictive productivity models	3.73	3.55	7.27
12	Depth and breadth of genomes	3.27	3.82	7.09
13	Predator collections	3.82	3.27	7.09
14	Efficient transformation protocols	3.45	3.64	7.09
15	Metabolomics tools	3.27	3.64	6.91
16	Algal-bacterial interactions	3.45	3.36	6.82
17	Mass balance closure	3.73	3.09	6.82
18	Analysis pipelines for meta-omics	3.27	3.45	6.73
19	Mating protocols	3.45	3.27	6.73
20	Workforce/human resources	2.91	3.82	6.73
21	Metabolic reconstruction networks (after good annotated genomes)	3.45	3.09	6.55
22	Species-specific promoters, introns, untranslated regions for controlled gene expression	3.27	3.18	6.45
23	Cost-benefit analysis of PBRs vs. open ponds	2.91	3.55	6.45
24	Curated flux balance analysis models for algal genomes	3.36	3.09	6.45
25	Commercially available pond population analysis	3.36	3.00	6.36
26	Consensus on production strain	3.45	2.82	6.27
27	High oil prices	4.45	1.73	6.18
28	Reference library for metabolites	2.91	3.18	6.09

The top three priority resources all fell within the category of reference tools. Certified reference materials, shared online tools and/or databases, and reference strains for different environmental conditions (such as high temperature and low temperature) were recognized as currently lacking. Other resources in the reference tool category included a library for metabolites, predatory collections, consensus on production strains, mass balance closure, and standards and compliance.

The next three top-priority resources all happened to fall within the cultivation category. Large-scale longitudinal testing, low-cost *in situ* sensors, and high-throughput methods for characterizing strains are all related to the growth of algae crops. Other resources that the group identified as priorities in advancing algal cultivation were commercially available pond population analysis, predatory treatment databases, predictive productivity models, and workforce development programs. The participants also noted that having a better understanding of microbial ecology (including algal-bacterial interactions) would aid in the further development of cultivation methods. Another comment asserted the value in establishing a clear cost-benefit analysis approach in techno-economic analyses to compare cultivation in PBRs to open pond settings at large scales.

Within the category of omics tools, group members noted R&D priorities, including improved annotation of genomes with both breadth and depth, metabolic reconstruction networks, and curated flux balance analysis models. Participants also mentioned a need for analysis pipelines for meta-omics and improved metabolomics tools. Resources identified in the genetics tools category included mating protocols (breeding), efficient transformation protocols, as well as species-specific promoters, introns, and untranslated regions for controlled gene expression.

This breakout group also discussed the role of factors outside the control of the algal biology community. Both high oil prices and investment in further R&D, identified as "sheer luck" resources, were noted to play a role in the near-term advancement of the field.

Conclusion

The Algal Biology Toolbox Workshop gathered 62 experts in the field of algal biology to discuss new developments in algal biology and identify challenges and strategies to achieve production goals. Based on the presentations made by algal biology experts and the input received from stakeholders, there are several recommended areas of focus as the field moves forward toward producing commercially viable biofuel and bioproducts from algae. Areas of extensive discussion over the 2-day workshop included the need for further development of scientific tools, the prioritization of attributes for strain improvement efforts, and the remaining challenges for effective large-scale algae cultivation.

The algal biology toolbox encompasses the fundamental understanding of algal physiology and metabolism, the molecular tools and methodology required to optimize algal biology, the analytical tools required to characterize and study algae, omics knowledge and development, and the reference databases utilized for tool development and data sharing. Discussing the need for further development of tools, the presenters from day one noted that the field would benefit from increased coordination and collaboration among scientists working on multi-omics experimentation. A growing area that has a need for ongoing work is metabolomic profiling that provides insights toward the development of novel co-products. The refinement of genomic manipulation and editing tools may assist with these efforts as well as other ongoing multi-omics experiments. There is also room for improvement in sequencing platforms, especially in the development of portable technologies that can be used on location. Challenges also remain in the annotation of algal genes and algal genome assembly. There are existing genome databases, but the quality of these tools needs to be improved so that algal data is more actionable. Building metabolic models for predictive learning applications would also be valuable in this regard. Lastly, presenters commented that there are basic husbandry approaches (breeding) that can increase algal yields and that there is room to improve the use of genetic modification and knockdown of native genes as genetic tools that are not defined as genetic modification and, therefore, are not subject to regulation.

Presenters also discussed the traits that are being targeted for algal strain improvement. Experts noted that the methodology and approach used depends on the desired traits and targeted use. The following are some of the traits noted:

- High biomass productivity

- Increased oil yield

- Photosynthetic efficiency

- Ease of harvesting

- Broad temperature and oxygen tolerance

- High photosynthetic capacity

- Contamination resistance

- Non-toxicity

- Non-invasiveness.

Presenters also discussed the need for consistency and repeatability, which has proved to be a challenge, especially in scaling experimental strains.

Addressing next steps in cultivation improvements, experts noted that outdoor cultivation is necessary in order to observe characteristics such as compositional rearrangement, clumping, and settling. Participants recommended

that laboratory biologists partner with outdoor testbeds in order to apply their experimental approaches to production environments. Also noted was the need for cultivation experiments to be benchmarked consistently, measuring productivity with uniform and standardized language. Another suggestion was to co-culture algae with known predators in order to establish a matrix of cultivation treatment options. Finally, the field will need to figure out how to best harvest secreted products.

A primary objective for DOE in hosting the Algal Biology Toolbox Workshop was to gather input from expert stakeholders about the critical next steps in developing algal biofuels and bioproducts. The facilitated breakout sessions provided valuable comments in this regard. In general, the feedback from participants at this event can be categorized into the following focus areas:

- Understanding the fundamental physiology and metabolic processes related to carbon flux and valuable product formation

- Developing, integrating, and annotating omics datasets

- Creating robust genetic transformation and genome editing tools

- Understanding and exploiting algal breeding and adaptive evolution systems

- Characterizing the microbial ecology of algal cultures

- Developing high-throughput screening and analytical tools that can select not only for traits of interest (such as high lipid accumulation), but also for the ability to grow to high productivity outdoors at larger and larger scales.

BETO would like to express our thanks to all of the participants for their time, efforts, and contributions. The discussions and information provided through the plenary presentations, open forum, and breakout sessions are extremely valuable to the Advanced Algal Systems Program, and we look forward to continued collaboration as we utilize this feedback to inform program strategies moving forward.

Appendix A: Workshop Agenda

Sharpening Our Tools: Algal Biology Toolbox Workshop
U.S. Department of Energy, Bioenergy Technologies Office
Hard Rock Hotel San Diego
207 Fifth Ave., San Diego, CA 92101

Tuesday, May 24, 2016
Day 1 Presentations – New Developments in Algal Biology
Purpose: Experts in the field of algal biology will share their recent successes with participants to further the community's understanding of the current state of the art. These presentations will ensure that a range of expertise is represented at the event.

Time	Agenda Item	Speaker Name
8:00 a.m. – 8:05 a.m.	Welcome and Introductions	Alison Goss Eng, Bioenergy Technologies Office
8:05 a.m. – 8:20 a.m.	Introduction: New Discoveries in Biology and Biochemistry	Amanda Barry, Bioenergy Technologies Office
8:20 a.m. – 8:50 a.m.	Molecular Toolbox Development and Gene Expression	Matt Posewitz, Colorado School of Mines
8:50 a.m. – 9:20 a.m.	Molecular Toolbox Development and Gene Expression	Mark Hildebrand, University of California, San Diego
9:20 a.m. – 9:30 a.m.	Break	
9:30 a.m. – 10:00 a.m.	Omics Databases	Shawn Starkenburg, Los Alamos National Laboratory
10:00 a.m. – 10:30 a.m.	Omics Databases at the Joint Genome Institute	Igor Grigoriev, Joint Genome Institute
10:30 a.m. – 11:00 a.m.	Gene Expression and Silencing	Heriberto Cerutti, University of Nebraska, Lincoln
11:00 a.m. – 11:30 a.m.	Co-Products Value Proposition	Lieve Laurens, National Renewable Energy Laboratory
11:30 a.m. – 12:00 p.m.	Novel Co-Product Concepts	Stephen Mayfield, University of California, San Diego
12:00 p.m. – 1:00 p.m.	Lunch	
1:00 p.m. – 1:20 p.m.	From Flask to Farm	Taraka Dale, Los Alamos National Laboratory
1:20 p.m. – 1:40 p.m.	From Flask to Farm	Phillip Pienkos, National Renewable Energy Laboratory
1:40 p.m. – 3:40 p.m.	Biological Improvements in Cultivation	Chris Yohn, Sapphire Energy Harlan Miller, Algenol Dave Hazlebeck, Global Algae Innovations Henry Taunt, Algenuity Richard Sayre, Los Alamos National Laboratory Zackary Johnson, Duke University (20 minutes each)

Time	Agenda Item	Speaker Name
3:40 p.m. – 3:50 p.m.	Break	
3:50 p.m. – 4:50 p.m.	Open Forum Discovery Presentations *Five-minute briefings from participants on topics of their interest, suggested focus on achievements to date, current barriers, and outlook for the future of algal biology*	Open to registrants
4:50 p.m. – 5:00 p.m.	Closing Summary for Day 1	

Wednesday, May 25, 2016
Day 2: Facilitated Breakout Discussions – Identifying Challenges and Strategies
Purpose: To provide a forum to discuss our understanding of algal biology and biochemistry, available molecular toolboxes, omics databases, and other resources to identify challenges and to measure our progress toward commercial algal biofuels.

Time	Agenda Item	Speaker Name
8:00 a.m. – 8:15 a.m.	Convene: Review of Breakout Session Format and Objectives	Alison Goss Eng, Bioenergy Technologies Office
8:15 a.m. – 9:45 a.m.	Breakout Session #1: Biology Unknowns	
9:45 a.m. – 10:15 a.m.	Plenary Report Out from Breakout Session #1	
10:15 a.m. – 10:30 a.m.	Break	
10:30 a.m. – 12:00 p.m.	Breakout Session #2: Species of Focus	
12:00 p.m. – 12:30 p.m.	Plenary Report Out from Breakout Session #2	
12:30 p.m. – 1:30 p.m.	Lunch	
1:30 p.m. – 3:00 p.m.	Breakout Session # 3: Future Directions	
3:00 p.m. – 3:30 p.m.	Plenary Report Out from Breakout Session #3	
3:30 p.m. – 3:45 p.m.	Summary Conclusions and Thank You to Participants	Amanda Barry, Bioenergy Technologies Office

Appendix B: Breakout Session Questions

Breakout Session #1: Biology Unknowns

- What are challenges in algal biology, specific to genetic tools, that pose significant hurdles to progress over the next 5 years?

- Brainstorm, categorize, and prioritize challenges in algal biology.

Breakout Session #2: Species of Focus

- What does success look like; what attributes would the ideal commercial strain(s) possess?

- What traits should we target for engineering?

- How have existing production strains performed against these attributes?

- What metrics should we use to measure progress in these attributes?

- Rank ideal strain attributes according to importance in near-term advancement of commercial purposes.

Breakout Session #3: Future Directions

- What tools and resources do we have at our disposal to overcome the challenges from session #1 and to achieve the ideal strains from session #2?

- What tools and resources are still needed?

- Brainstorm, categorize, and prioritize tools and resources needed to overcome near-term challenges in algal biology.

Appendix C: Abbreviations and Acronyms

BETO   Bioenergy Technologies Office

DNA   deoxyribonucleic acid

DOE   U.S. Department of Energy

EERE   Office of Energy Efficiency and Renewable Energy

LANL   Los Alamos National Laboratory

MAGIC   Marine Algae Industrialization Consortium

NREL   National Renewable Energy Laboratory

PBR   photobioreactor

R&D   research and development

RNA   ribonucleic acid

rRNA   ribosomal ribonucleic acid

Appendix D: Related Links

Algal Biofuels Strategy Workshops

energy.gov/eere/bioenergy/algal-biofuels-strategy-workshops

National Algal Biofuels Technology Review

energy.gov/sites/prod/files/2016/06/f33/national_algal_biofuels_technology_review.pdf

Bioproducts to Enable Biofuels Workshop

energy.gov/eere/bioenergy/bioproducts-enable-biofuels-workshop-0

Bioproducts to Enable Biofuels Workshop Summary Report

energy.gov/sites/prod/files/2015/12/f27/bioproducts_to_enable_biofuels_workshop_report.pdf

Bioenergy Technologies Office

bioenergy.energy.gov

Bioenergy Technologies Office Multi-Year Program Plan: March 2016

energy.gov/eere/bioenergy/downloads/bioenergy-technologies-office-multi-year-program-plan-march-2016

Office of Energy Efficiency and Renewable Energy

energy.gov/eere/office-energy-efficiency-renewable-energy

U.S. Department of Energy

energy.gov

Appendix E: Workshop Participants

The following is a list of participants who elected to share their contact information in these proceedings:

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