U.S. Department of Energy
Advanced Research Projects Agency-Energy (ARPA-E)

Request for Information
DE-FOA-0003470
on Plant Genetic Engineering for Energy Applications

Introduction:

The purpose of this Request for Information (RFI) is to solicit input for a potential ARPA-E program focused on the development of tools to enable plant genetic engineering and synthetic biology. The goals for this potential program include the evaluation of technologies capable of:

1. Accelerating plant genetic modifications;
2. Increasing the range of genetic modifications possible; and
3. Decreasing the variability and reducing the difficulty of regenerating plants after genetic modifications.

ARPA-E seeks input from plant scientists, plant geneticists, molecular biologists, synthetic biologists, crop breeders, and robotics and machine learning engineers. Additionally, ARPA-E seeks input from other stakeholders in the plant biotechnology ecosystem, including seed producers, growers, plant transformation centers, and biotechnology companies. The questions listed are intended to assist relevant stakeholders in providing input on:

- Technological approaches to accelerating plant genetic engineering for traits of relevance to ARPA-E's statutory goals;
- Success metrics to quantify the impact of new technological approaches to plant genetic engineering on innovation and discovery, trait development, and commercialization of improved crop varieties;
- Approaches to evaluating the technical feasibility of these strategies; and
- Factors for widespread adoption of these new technologies.

Areas Not of Interest for Responses to this RFI:

- Work focused on basic research aimed purely at discovery and fundamental knowledge generation;
- Land management or agronomic practices; and
- Microbial approaches to engineer soil or plant microbiomes.

RFI Guidelines:

CAREFULLY REVIEW ALL RFI GUIDELINES BELOW.

Note that the information you provide will be used by ARPA-E solely for program planning, without attribution. **THIS IS A REQUEST FOR INFORMATION ONLY. THIS RFI DOES NOT CONSTITUTE A FUNDING OPPORTUNITY. NO FUNDING OPPORTUNITY EXISTS AT THIS TIME.**

The purpose of this RFI is solely to solicit input for ARPA-E's consideration to inform the possible formulation of future research programs. ARPA-E will not provide funding or compensation for any information submitted in response to this RFI, and ARPA-E may use information submitted to this RFI without any attribution to the source. This RFI provides the broad research community with an opportunity to contribute views and opinions.

No material submitted for review will be returned and there will be no formal or informal debriefing concerning the review of any submitted material. ARPA-E may contact respondents to request clarification or seek additional information relevant to this RFI. All responses provided will be considered, but ARPA-E will not respond to individual submissions or publish publicly a compendium of responses. **Respondents shall not include any information in the response to this RFI that could be considered proprietary or confidential**.

Responses to this RFI should be submitted in PDF format to the email address **ARPA-E-RFI@hq.doe.gov** by **5:00 PM Eastern Time on November 11, 2024.** Emails should conform to the following guidelines:

- Insert "<your organization name> - Response to RFI on Plant Genetic Engineering" in the email subject line.
- In the body of your email, include your name, title, organization, type of organization (e.g., university, non-governmental organization, small business, large business, federally funded research and development center [FFRDC], government-owned/government-operated [GOGO]), email address, telephone number, and area of expertise.
- Responses to this RFI are limited to no more than 10 pages in length (12-point font size).
- Responders are strongly encouraged to include preliminary results, data, and figures that describe their potential materials, designs, or processes.

Technical Background:

Plants play a crucial role in ARPA-E's statutory goals through various applications. These include the production of biofuels, the creation of chemicals and materials (e.g., polymers and building materials) that can replace petroleum-based products, the capture and utilization of atmospheric carbon dioxide, and the accumulation of critical minerals through phytomining. Breeding and management practices have led to dramatic improvements in agriculture with crop yields increasing by 135% from 1961 to 2005.[1] However, U.S. yields are projected to increase more slowly (e.g., corn) or even decline (e.g., soybeans) over the next decade due to climate change.[2] Meanwhile, demand for biofuels is increasing to meet the growing market for Sustainable Aviation Fuel (SAF). The Department of Energy's SAF Grand Challenge has set a SAF production target of 35 billion gallons per year by 2050, which requires 60 billion gallons of ethanol to meet the target due to ethanol's lower energy density.[3,4] This is more than three

[1] Jennifer A. Burney, Steven J. Davis, and David B. Lobell, "Greenhouse Gas Mitigation by Agricultural Intensification," *Proceedings of the National Academy of Sciences* 107, no. 26 (June 15, 2010): 12052–57, https://doi.org/10.1073/pnas.0914216107.

[2] Jayson Beckman, Maros Ivanic, and Noé J. Nava, "Estimating Market Implications From Corn and Soybean Yields Under Climate Change in the United States," October 1, 2023, https://doi.org/10.32747/2023.8134358.ers.

[3] U.S. Department of Energy, "SAF Grand Challenge Roadmap," report, *SAF Grand Challenge Roadmap*, n.d., https://www.energy.gov/sites/default/files/2022-09/beto-saf-gc-roadmap-report-sept-2022.pdf.

[4] United Nations International Civil Aviation Organization, "SAF Rules of Thumb," n.d., https://www.icao.int/environmental-protection/Pages/SAF_RULESOFTHUMB.aspx.

times the combined production of ethanol and biodiesel in 2022, so breeding and management are unlikely to meet the challenge.[5] Moreover, some of the greatest opportunities for plants to impact energy involve traits that do not currently exist within the plant kingdom, such as nitrogen fixation and the accumulation of polymer precursors.[6,7]

Plant genetic engineering is a complementary strategy that may transform agriculture into a sustainable source of energy and materials. A typical plant genetic engineering workflow is illustrated in Figure 1 and involves the following steps: 1) transformation of DNA into undifferentiated plant cells known as calli, 2) regeneration of the cells into a plantlet via tissue culture, and 3) transplantation of the plantlet into soil in a greenhouse. From there, seeds can be grown, or 4) explant and callus can be generated to iterate. It is also necessary to prepare the transgene and vector (step 0) before transformation.

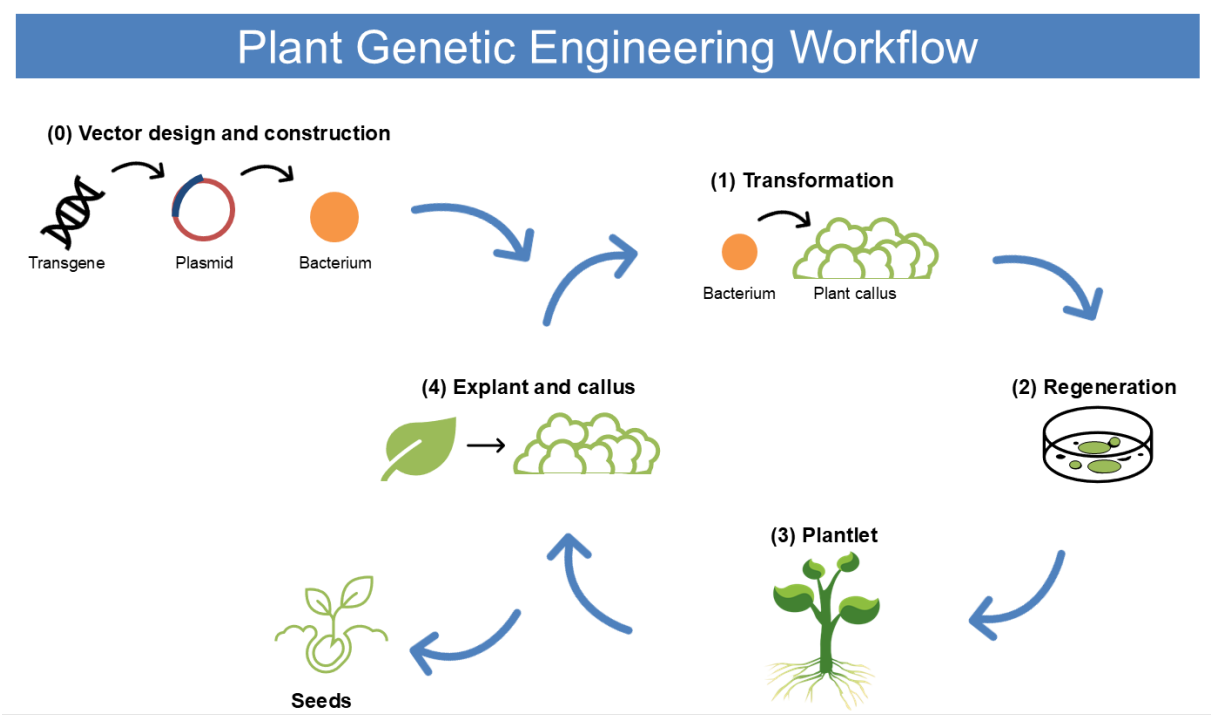

Figure 1. A typical plant genetic engineering workflow.[8]

[5] United States Department of Agriculture Economic Research Service, U.S. Bioenergy Statistics, n.d., https://www.ers.usda.gov/data-products/u-s-bioenergy-statistics/.

[6] Robert S. Allen et al., "Expression of 16 Nitrogenase Proteins Within the Plant Mitochondrial Matrix," *Frontiers in Plant Science* 8 (March 3, 2017), https://doi.org/10.3389/fpls.2017.00287.

[7] Chien-Yuan Lin et al., "In-planta Production of the Biodegradable Polyester Precursor 2-pyrone-4,6-dicarboxylic Acid (PDC): Stacking Reduced Biomass Recalcitrance With Value-added Co-product," *Metabolic Engineering* 66 (July 1, 2021): 148–56, https://doi.org/10.1016/j.ymben.2021.04.011.

[8] Adapted from Walter Suza and Donald Lee, "Chapter 12: Genetic Engineering" in *Genetics, Agriculture, and Biotechnology*, 2021, https://doi.org/10.31274/isudp.2021.113.

Depending on the cultivar, the time from explant generation to seeds can take 12-16 months. Plant genetic engineering has been practiced since the 1980s, but existing methods suffer from major limitations in speed, flexibility, and robustness (Table 1).[9]

To realize the full potential of plants and agriculture in energy, ARPA-E seeks information on technologies to accelerate and improve plant genetic engineering. ARPA-E is interested in technologies to rapidly generate and test genetic modifications in a wide variety of plant cultivars, unlocking the iterative design-build-test-learn loops that have enabled rapid innovation in other engineering fields. Interest is given to non-model organisms that can be deployed in the field for energy applications. ARPA-E is requesting information to evaluate approaches meeting the goals of this programmatic concept.

Table 1. Limitations of existing tools for plant genetic engineering.

Technique	Limitations
***Agrobacterium* transformation**	Efficiency, host range, payload size, random integration
Regeneration	Speed (months to >1 year), labor, efficiency, reproducibility, generalizability
Gene insertion (homology-directed repair, prime editing, transposase)	Efficiency, gene size

RFI Questions:

The questions posed in this section are organized into several different groups. Respondents may provide responses and information about any of the following questions. **ARPA-E does not expect any one respondent to answer all, or even many, of the questions in this RFI.** In your response, indicate the question number (e.g., Response to RFI Question 1.a). Appropriate citations are highly encouraged. Respondents are also welcome to address other relevant avenues or technologies that are not outlined below, except for those that fall under the "Areas Not of Interest for Responses to this RFI" described above.

1. Priorities for plant genetic engineering for energy applications

The plant varieties and traits most relevant for ARPA-E's statutory goals may differ substantially from those that have been optimized for food production. For this potential program, ARPA-E seeks to identify the specific plant species, varieties, traits, and pathways most relevant to our statutory goals, and to determine how these targets shape the needs for tool development.

a. What crops and traits are highest priority to engineer to achieve the following goals?
    i. Biofuel production
    ii. Production of chemicals, polymers, and other materials
    iii. Carbon sequestration in biomass and in associated soils
    iv. Phytomining

[9] Mieke Van Lijsebettens and Geert Angenon, "Thirty Years of Transgenic Research in Plants," *The International Journal of Developmental Biology* 57, no. 6-7–8 (January 1, 2013): 445–47, https://doi.org/10.1387/ijdb.130062mv.

b. What other crops and traits are likely to impact ARPA-E statutory goals in energy production and mitigate greenhouse gas emissions at meaningful scale?
c. How do the non-food priorities above change the goals for tool development relative to food traits?

2. State of the art and objectives

ARPA-E seeks to understand and quantify the state of the art (SoA) for plant genetic engineering.

a. What metrics are most relevant for plant genetic engineering workflows? For specific crops, what is the SoA for these metrics? What improvements are likely in the near term?
b. What are the biggest bottlenecks to achieving transformational improvements to the SoA? What impact would result from overcoming these bottlenecks?
c. Fill out the following table for specific crops with which you are familiar. Here, "event" is defined as a desired change to the genome recovered in a mature plant. A "full-time equivalent" (FTE) is the number of full-time employees or students necessary to produce a result. In your response, specify the setting (e.g., academic lab, start-up, transformation facility) and perspective (e.g., the respondent's lab, the industry average, a best-in-class facility).

Crop/variety	Capacity: Number of events per year	Total time to generate an event	FTEs to generate an event	Approximate cost to generate an event	Comments

d. For the above crops, what improvement in capacity would revolutionize plant genetic engineering workflows? What new capabilities would such a capacity unlock? What could be achieved with this increased capacity that is not available today?
e. What improvement in capacity is achievable within a three-year ARPA-E program?
f. What crops and varieties can be routinely transformed by individual academic labs and by academic plant transformation centers? What crops and varieties can be routinely transformed by large seed companies?
g. What is the availability of plant transformation? Do you perform transformation in your own lab, at a nonprofit plant transformation center, or through a private entity? Does transformation capacity meet demand? How expensive is plant transformation?

3. Transformation and regeneration

Many plant genetic engineering workflows can be divided into the introduction of genetic material into a plant cell (transformation) and the regeneration of a whole plant, often via tissue culture (see Figure 1). ARPA-E seeks to understand the SoA for transformation and regeneration as well as opportunities to disrupt this paradigm.

a. Transformation
    i. For the following technologies, describe SoA efficiencies, the limitations of the SoA, and opportunities for disruptive improvements:

- *Agrobacterium*-mediated transformation
- Biolistic transformation
- Nanoparticle transformation
- Electroporation
- Transformation with viral vectors
- Protoplast transformation

ii. What other technologies offer the potential for disruptive improvements in transformation? What are the most promising technologies?

iii. What is the SoA for transforming large DNA constructs? What is the largest size of DNA that can be transformed routinely? What is the largest size that can be transformed in best-of-class experiments?

iv. How can 100 kilobase (kb) DNA constructs be transformed? How can DNA constructs that are one megabase (Mb) and larger be transformed? What new capabilities would transformation of DNA constructs of these sizes unlock?

v. What are the theoretical explanations of the substantial differences between transformation efficiency within a single species? How could engineering narrow these differences within a species? How important would it be to narrow such differences?

b. Regeneration

i. For specific crops with which you are familiar, what is the process of regenerating a plant via tissue culture? How long does this process take? How does that length compare to the maturation process of a wild type seedling?

ii. How long does it take to generate a fragment of plant tissue amenable to transformation?

iii. How does selection influence the regeneration process? Is the availability of selectable markers a bottleneck?

iv. What are the opportunities associated with morphogenic genes? How much can regeneration be improved using morphogenic genes? What are the current constraints on the availability of morphogenic gene technology for transformation?

v. Are pollen gametes a plausible transformation target? Would the transformation of pollen reduce the regeneration penalty?

vi. What other bottlenecks and opportunities exist for regeneration?

c. Which of the following is most impactful for plant genetic engineering: improvements to transformation, improvements to regeneration, or something else?

d. Are there other workflows, like floral dipping, that break the paradigm of transformation/regeneration? How could floral dipping be extended to a broader range of species? What other opportunities exist to bypass regeneration/tissue culture altogether?

4. Other tools

For this potential program, ARPA-E seeks to understand the SoA and potential for disruption of other tools for plant genetic engineering. Targeted gene insertion remains difficult in plants due to the low efficiency of homology-directed repair (HDR).[10] However, since the advent of the CRISPR-Cas9 system, diverse molecular tools have been successfully applied to plants. For example, prime editors have been shown to insert up to 34 base pairs and transposase-based systems have been used to perform targeted

[10] Carla Schmidt et al., "DNA Break Repair in Plants and Its Application for Genome Engineering," *Methods in Molecular Biology*, November 11, 2018, 237–66, https://doi.org/10.1007/978-1-4939-8778-8_17.

insertions of 8.6 kb in *Arabidopsis*.[11,12] Concurrently, artificial intelligence and machine learning (AI/ML) are delivering increased predictive power in all areas of biology, including the critical task of predicting genotype-phenotype relationships.[13] On the hardware side, new single-cell methods have begun to enable high-throughput assays on previously impractical scales.[14] None of these tools are expected to be panaceas for the challenges of plant genetic engineering, but ARPA-E seeks to understand their potential to deliver disruptive new capabilities in combination with each other and with other advances in transformation/regeneration.

a. Molecular/genomic tools
    i. For the following technologies, describe efficiencies, limitations, and opportunities for disruptive improvements:
        - CRISPR-mediated gene insertion
        - Prime editing
        - Transposon-based gene insertion
    ii. What other opportunities exist for improving gene insertion (e.g., size, targeting, regulation)?
    iii. What are the prospects for utilizing the HDR pathway for gene insertion?
    iv. What other molecular or genetic tools have the potential to impact genetic engineering?
b. Hardware (robotic/mechanical) and software tools
    i. What opportunities exist to utilize AI/ML for disruptive innovation in plant engineering? What biological or hardware tools are necessary to unlock this potential? What role can computer vision play in this space?
    ii. What opportunities exist to utilize automation and robotics for plant engineering? How can the accessibility of such tools be expanded to academic labs and nonprofit transformation centers?
    iii. What opportunities exist for new hardware to physically manipulate cells (e.g., tissue culture, cell culture, bioreactors)? Are there opportunities for single-cell workflows and phenotyping?
    iv. What other hardware and software tools have the potential to create a disruptive impact in plant engineering?
c. Are there any tools or methods not listed above which have the potential to make a disruptive impact in plant engineering?

[11] Yuan Zong et al., "An Engineered Prime Editor With Enhanced Editing Efficiency in Plants," *Nature Biotechnology* 40, no. 9 (March 24, 2022): 1394–1402, https://doi.org/10.1038/s41587-022-01254-w.
[12] Peng Liu et al., "Transposase-assisted Target-site Integration for Efficient Plant Genome Engineering," *Nature*, June 26, 2024, https://doi.org/10.1038/s41586-024-07613-8.
[13] Chia-Yi Cheng et al., "Evolutionarily Informed Machine Learning Enhances the Power of Predictive Gene-to-phenotype Relationships," *Nature Communications* 12, no. 1 (September 24, 2021), https://doi.org/10.1038/s41467-021-25893-w.
[14] Rachel Shahan et al., "A Single-cell Arabidopsis Root Atlas Reveals Developmental Trajectories in Wild-type and Cell Identity Mutants," *Developmental Cell* 57, no. 4 (February 1, 2022): 543-560.e9, https://doi.org/10.1016/j.devcel.2022.01.008.

5. Adoption, enabling conditions, and technology-to-market

New tools for plant genetic engineering will fail to have an impact on ARPA-E's statutory goals unless they are widely adopted. The history of gene editing provides an illustrative example. Abstractly, zinc finger proteins (ZNFs), transcription activator-like effector nucleases (TALENS), and CRISPR-Cas9 all provide similar gene editing capabilities. However, CRISPR's ease-of-use has made it the tool of choice for most applications and has democratized access to gene editing, leading to massive downstream impacts.[15] In contrast, the protocols for plant transformation and regeneration are notoriously fragile, with substantial variability in outcomes between users. Moreover, intellectual property and regulatory issues will play a major role in commercializing any tools or engineered plants. For this potential program, ARPA-E seeks to understand the factors affecting tool adoption and commercialization to maximize the impact of this programmatic concept.

a. Adoption
    i. What properties of a new tool or method enable widespread, rapid adoption?
    ii. How can a new tool achieve reproducibility, robustness, and ease of use?
    iii. How can inter-person and inter-lab variability in results and efficiencies be reduced?
    iv. What considerations impact tool adoption by academics? What considerations impact adoption by industry?
    v. How does the availability of biological reagents affect tool adoption? How does the availability of physical hardware affect adoption?
    vi. How do intellectual property and licensing considerations affect tool adoption?
b. Commercialization
    i. What is the path to commercialization of a tool for plant genetic engineering?
    ii. What is the path to commercialization for a genetically modified plant that is developed in an academic lab? Does this differ between common crops (e.g., corn, soybeans) and emerging crops for biofuel production (e.g., sorghum, miscanthus)?
    iii. How do regulatory considerations impact the plant genetic engineering innovation pipeline? How do academic labs perform small-scale field trials of modified plants?

[15] Anuradha Bhardwaj and Vikrant Nain, "TALENs—an Indispensable Tool in the Era of CRISPR: A Mini Review," *Journal of Genetic Engineering and Biotechnology* 19, no. 1 (August 21, 2021): 125, https://doi.org/10.1186/s43141-021-00225-z.