Environmental Biotechnology

EBRC Engineering Biology Research Consortium

5885 Hollis Street, 4th Floor, Emeryville, CA 94608
Phone: +1.510.871.3272   Fax: +1.510.245.2223

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Environmental Biotechnology focuses on the technologies and tools to enable deployment of bioengineered systems in the land, air, water, and human landscapes for purposes related to remediation, natural resource management, environmental monitoring, and species management. In contrast to other sectors - including industry and agriculture - this technology is deployed in the environment in a process design that is inherently poorly controlled. Technical and societal challenges therefore reflect its broad and uncontrolled nature. The Challenges, Aims, and Objectives in this sector are highly parallel to those that also appear in *Energy* and *Health & Medicine*.

Societal Challenge 1: Address and mitigate climate change.

- Science/Engineering Aim 1: Enable adaptation of ecosystems to climate change.
    - Engineering Biology Objective 1: Enable and advance the production of drought-tolerant vegetation for growth on marginal land.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Genetic tools to edit non-model plants, including trees.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Plants engineered with waxy leaves to prevent evaporation.
            - Physiological root mechanisms for concentrating water and functioning in high-ionic-strength soils.
            - Engineer C4 pathways into C3 plants for increased drought- and heat-resistance.
        - Host and Consortia Engineering Achievement:
            - Transcription factor engineering to turn on/off water stress-related pathways.
            - Introduction of heterologous genes from other plants that enable moisture sensing (e.g., transporters, etc.).
    - Engineering Biology Objective 2: Enable and advance the production of self-fertilizing plants and/or cover crops.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Complex genotype construction in both model (e.g., maize) and non-model (e.g., clover, vetch) plants.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Optimize nitrogen fixation pathway enzymes to fit new host organisms.
        - Host and Consortia Engineering Achievement:
            - Transfer the nitrogen fixation pathway into plant.
            - Increase the nitrogen fixing activity of rhizobia-containing plants.
        - Data Integration, Modeling, and Automation Achievement:
            - Accurate prediction of nitrogen fixation pathways to insert into plant.
            - Multi-organism/multi-scale modeling of element cycling.

- Engineering Biology Objective 3: Engineering biomes for robust soils.
    - Gene Editing, Synthesis, and Assembly Achievement:
        - Genome engineering capabilities for implementing large numbers of targeted modifications in specific hosts that may have limited tools for transformation, modification, and programmable gene expression.
        - Genetic tools to engineer a variety of soil microbes.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
        - Soil microbes engineered to produce extracellular polymers that stabilize the soil.
        - Biosensing systems to sense phosphate/nitrogen levels.
        - Engineered extracellular/intracellular mechanisms to concentrate critical elements and promote their long-term storage and/or release in soil, rather than dissolution and field run-off.
        - Engineer stable production of antibiotic and antifungal compounds to provide disease suppression for newly established crop plants.
    - Host and Consortia Engineering Achievement:
        - A variety of soil microbes that can be readily engineered (including transformation).
        - Produce stable, engineered microbial cultures in an agriculture setting.
        - Engineer microbial communities to sense water, carbon, and other nutrient contents of soils and secrete/consume appropriate nutrients to maintain the soil.
    - Data Integration, Modeling, and Automation Achievement:
        - Analysis of stability of engineered microbes in the soil.
        - Better understanding (analysis, modeling, and prediction) of microbial consortia in natural systems and how they interact and evolve over time and under different conditions.

- Science/Engineering Aim 2: Enable and advance carbon sequestration from the environment.
    - Engineering Biology Objective 1: Engineer soil biomes that more efficiently sequester carbon.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Genetic tools to engineer a variety of soil microbes.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Development of biopolymers that are not readily biodegraded and that can be secreted by engineered organisms into soil.
        - Host and Consortia Engineering Achievement:
            - Plants engineered to secrete carbonaceous materials into the soil for long-term carbon sequestration.
            - Microbes engineered to secrete recalcitrant biopolymers to extend sequestration periods.

- Data Integration, Modeling, and Automation Achievement:
  - Environmental-scale modeling of biome population dynamics.
- Engineering Biology Objective 2: Engineer highly productive plants for improved CO_2 removal and recycling from the environment.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Edit genes in the photosynthetic pathway for improved properties, including stability, catalytic activity, and substrate specificity.
  - Biomolecule, Pathway, and Circuit Engineering Achievement:
    - Improve efficiency of key enzyme(s) in the photosynthetic pathway.
  - Host and Consortia Engineering Achievement:
    - Introduce synthetic (heterologous or modified) enzymes/complexes/pathways for photosynthetic efficiency, such as to increase wavelength absorption via engineered chromophores and to enable CO_2 concentrating mechanisms in C3 crops (i.e., C4-like or cyanobacterial-like mechanisms).
  - Data Integration, Modeling, and Automation Achievement:
    - Engineered-photosynthetic pathway modeling.
- Engineering Biology Objective 3: Engineer organisms for improved methane removal and/or recycling from the environment.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Improve genetics in methanotrophs.
  - Biomolecule, Pathway, and Circuit Engineering Achievement:
    - Improve heterologous expression of methane assimilation pathways.
    - Creation of non-natural pathways for methane utilization.
  - Host and Consortia Engineering Achievement:
    - Rapid genetic construction of methanotrophs.
    - Stable deployment of engineered methanotrophs in appropriate settings (such as well-heads).
  - Data Integration, Modeling, and Automation Achievement:
    - Predictive models of microbial ecology across a range of conditions and environments.
- Engineering Biology Objective 4: Engineer recalcitrant, sinking marine phytoplankton for long-term carbon storage.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Multi-gene modification in non-model algae and cyanobacteria.
  - Biomolecule, Pathway, and Circuit Engineering Achievement:
    - Redirection of photosynthate into recalcitrant biopolymers.
    - Enhance biological mechanisms for carbonate formation (i.e., fixing carbon in a non-reduced form).

                - Alter structural proteins and/or physiology in order to control phytoplankton density and subsurface deposition rate (i.e., sinking).
            - Host and Consortia Engineering Achievement:
                - Engineer hosts that, once they reach a certain density, aggregate/filament to accelerate sinking.
                - Reduce the need for iron, which is often limiting in the marine environment.
            - Data Integration, Modeling, and Automation Achievement:
                - Life cycle analysis of carbon turnover in the environment.

Societal Challenge 2: Expand tool sets for bioremediation and resource recycling.

- Science/Engineering Aim 1: Enable better, more advanced bioremediation of petrochemical pollutants including plastics.
    - Engineering Biology Objective 1: Engineer microorganisms to rapidly degrade hydrocarbons after an oil spill.
        - Gene Editing Achievement:
            - Engineer new genomic programs, such as combinations of synthetic auxotrophies, that increase the safety and reduce the risk of deploying engineered microbes in the field.
        - Biomolecular Engineering Achievement:
            - Synthesis of enzyme libraries to enable the identification of synthetic pathways with improved degradation performance.
            - Engineer improved transporters for hydrocarbon uptake.
            - Engineer catabolic enzymes with enhanced catalytic turnover.
            - Engineer orthogonal versions of critical enzymes that require xenobiotic molecules to function.
        - Host Engineering Achievement:
            - Hosts with improved tolerance to chemical insults.
            - Hosts that secrete engineered enzymes.
            - Hosts that selectively secrete natural, biodegradable detergents (e.g., bile salt-like detergents) to facilitate hydrocarbon turnover.
        - Data Integration, Modeling, and Automation Achievement:
            - Modeling of ecosystems (including ocean currents) to evaluate rapid deployment when necessary, such as post-oil spill.
    - Engineering Biology Objective 2: Engineer microorganisms to degrade recalcitrant plastics such as polyethylene terephthalate (PET).
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Engineer new genomic programs, such as combinations of synthetic auxotrophies, that increase the safety and reduce the risk of deploying engineered microbes in the field.

- Biomolecule, Pathway, and Circuit Engineering Achievement:
  - Synthesis of enzyme libraries to enable the identification of synthetic pathways with improved degradation performance.
  - Engineer enzymes that convert xenobiotic plastics into functional metabolic intermediates.
- Host and Consortia Engineering Achievement:
  - Hosts that secrete engineered enzymes.
- Data Integration, Modeling, and Automation Achievement:
  - Predictive modeling of ecosystem-wide ramifications of engineered-organism deployment into polluted ecosystems (including secondary/downstream and long-term effects) to evaluate regions of highest need and viability for organism deployment.

- Science/Engineering Aim 2: Improve bioremediation and revitalization of water and soil.
  - Engineering Biology Objective 1: Introduction of engineered microorganisms, plants, and animals (specifically fish and mussels) for watershed and wastewater remediation and revitalization.
    - Gene Editing, Synthesis, and Assembly Achievement:
      - Synthesis of gene clusters to produce relevant degradation machinery.
      - Strategies for efficient genome editing of naturally-occurring species to be re-introduced into the environment.
      - Engineer new genomic programs, such as combinations of synthetic auxotrophies, that increase the safety and reduce the risk of deploying engineered microbes in the field.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
      - Design of novel enzymes to degrade xenobiotic small molecules such as toxins and antibiotics.
      - Design and construction of novel physiological mechanisms for the sequestration and processing of nitrogen and phosphate in run-off.
    - Host and Consortia Engineering Achievement:
      - Reliable integration of relevant pathways to be stable against genetic mutation.
      - Strategies to ensure the non-pathogenicity of the host (to the environment, over the long term), including the introduction of kill switches to remove engineered organisms once their work is finished.
      - Develop defined consortia of microbes and algae to most productively revitalize water sources.

            - Data Integration, Modeling, and Automation Achievement:
                - Predictive ecological models of species interactions to minimize the risk of uncontrolled cell growth in the environment/ecosystems.
    - Engineering Biology Objective 2: Plant platforms that can be engineered for remediation of a range of contaminated environments.
        - Gene Editing Achievement:
            - Plants transformed with pathways and metabolisms that enable the uptake of targeted contaminants and that have clearly visible 'markers' for public surveillance (for example, colors that clearly mark the plant as being genetically modified, so as to prevent people from eating these plants).
        - Biomolecular Engineering Achievement:
            - Enzymes engineered for efficient bioconversion and/or biosequestration of environmental contaminants.
        - Host Engineering Achievement:
            - Engineered variants of plants used for phytoremediation, such as Indian mustard (*Brassica juncea L.*), Willow (*Salix* species), Poplar (*Populus deltoides*), Indian grass (*Sorghastrum nutans*), and Sunflower (*Helianthus Annuus L.*), that do not permit the flow of DNA into wild-type plants.
        - Data Integration, Modeling, and Automation Achievement:
            - Design and modeling of genetic programs for pathways and engineered metabolisms in select organisms for a wide range of targeted environmental contaminants.
- Science/Engineering Aim 3: Enable more efficient and advanced resource recovery.
    - Engineering Biology Objective 1: Enable biorecovery of rare earth metals.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Knockout and gain-of-function systems to validate pathways/components and operation of sequestration involved in metal solubilization.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Detailed kinetics and stoichiometry of sequestration mechanisms, including interactions with relevant metals and competing ions.
            - Engineer orthogonal versions of critical enzymes that require xenobiotic molecules to function.
        - Host and Consortia Engineering Achievement:
            - Demonstration of solubilization and sequestration in relevant mesocosm studies.
        - Data Integration, Modeling, and Automation Achievement:
            - Complete modeling on most effective ecosystem for deployment of the organism, such as in an open-release scenario or in a

contained-use scenario which might be local (such as localized bioreactors at the point of recycling).

Societal Challenge 3: Controlled deployment of engineered organisms to improve ecosystem biodiversity, robustness, and the well-being of inhabitants.

- Science/Engineering Aim 1: Improve engineering of select insects for safe, effective environmental deployment.
  - Engineering Biology Objective 1: Design and produce insects with safe and effective gene drives to combat the spread of vector-borne infectious diseases. (Bier, Harrison, O'Connor-Giles, & Wildonger, 2018; Gantz & Bier, 2015; Gantz et al., 2015; Kyrou et al., 2018)
    - Gene Editing, Synthesis, and Assembly Achievement:
      - Gene engineering capabilities for producing targeted sterility in insects/arachnids regardless of species.
      - Gene editing capabilities to enhance vector resistance to parasites.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
      - Improve ability to target vector reproductive capabilities without off-target effects and ride-along mutations.
    - Host and Consortia Engineering Achievement:
      - Ability to introduce genetically-encoded "kill switches" such as auxotrophies dependent on localized, environmentally-available compounds.
      - Ability to enhance host (vector) antibody production against specific pathogen antigens.
    - Data Integration, Modeling, and Automation Achievement:
      - Better predictive long-term environmental and disease models incorporating climate change data with sterile vector release programs.
      - Increase automation capabilities for gene editing and rearing large numbers of sterile vectors of different species.
  - Engineering Biology Objective 2: Characterize and engineer natural microorganisms to control insect populations. (J. A. Gilbert & Melton, 2018; National Research Council (US) Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment, 1989)
    - Gene Editing, Synthesis, and Assembly Achievement:
      - Engineer new genomic programs, such as combinations of synthetic auxotrophies, that increase the safety and reduce the risk of deploying engineered microbes in the field.
      - Knockouts to validate candidate gene(s) in *Wolbachia* (a common parasitic microbe) to confirm genotype-to-phenotype models, to

use *Wolbachia* as a biological tool for controlling insect populations.

- Biomolecule, Pathway, and Circuit Engineering Achievement:
  - Identify key biomolecular interactions involved in host-microbe interactions to prepare for future engineering.
- Host and Consortia Engineering Achievement:
  - Validate function of candidate native microbial organisms.
  - Genome engineering capabilities for implementing large numbers of targeted modifications in specific hosts that may have limited tools for transformation, modification and programmable gene expression.
- Data Integration, Modeling, and Automation Achievement:
  - Genotype-to-phenotype tools for what makes *Wolbachia* function for controlling vector reproduction/fitness and other physiological functions.
  - Genotype-to-phenotype tools for identifying candidate organisms from natural populations.
  - Automation in rearing/sexing of infected organisms to fit use requirements.

- Engineering Biology Objective 3: Increase diversification and resilience of capable insect pollinators.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Knockout or gain-of-function systems to validate semiochemical attractant/repellent pathways in candidate animals.
    - Identification of genes involved in susceptibility to various pollinator pathogens and/or toxins (e.g., honey bee susceptibility to *Varroa* mites).
  - Biomolecule, Pathway, and Circuit Engineering Achievement:
    - Ability to affect multiple genes, with minimal off-target physiological effects, to achieve increased resistance to pollinator pathogens and toxins.
  - Host and Consortia Engineering Achievement:
    - Demonstrate functional circuits conferring host-insect attraction in engineered insects.
    - Validate function, viability, and behavior of candidate native organisms.
  - Data Integration, Modeling, and Automation Achievement:
    - Accurate prediction of plant-insect pairs and potential ecological off-target pairs.
    - Increased automation capabilities for gene editing and rearing large numbers of disease-resistant pollinators.

- Science/Engineering Aim 2: Improved engineering of select plants and animals for safe, effective, environmental deployment.
    - Engineering Biology Objective 1: Design and produce animals with effective gene drives to produce sterile animals to control invasive species populations. (Grunwald et al., 2019; Jones et al., 2016)
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Gene engineering capabilities for producing targeted sterility regardless of species.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Improved ability to target reproductive capabilities without off-target effects and ride-along mutations.
        - Host and Consortia Engineering Achievement:
            - Ability to introduce genetically-encoded “kill switches” such as auxotrophies dependent on localized, environmentally-available compounds.
        - Data Integration, Modeling, and Automation Achievement:
            - Better predictive long-term environmental and population models incorporating climate change data and engineered organism behavior/spread.
    - Engineering Biology Objective 2: Design and produce bio-containable engineered plant species. (National Research Council (US) Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment, 1989)
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Develop and expand transformation tools and sequence databases to support editing in plants of interest.
            - Further develop and enhance cytoplasmic male sterility in select species.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Enable biocontainment through mitochondrial and chloroplast engineering.
        - Host and Consortia Engineering Achievement:
            - Develop *Agrobacterium* strain(s) capable of transforming new plants of interest at workable efficiency.
        - Data Integration, Modeling, and Automation Achievement:
            - Better predictive long-term environmental and population models incorporating climate change data and engineered organism behavior/spread.

- Engineering Biology Objective 3: Use extant species homologues and genetic information for archiving and to achieve de-extinction for *select*, recently extinct (i.e., extinct for fewer than 150 years) species that have viable, available habitats.
  - Gene Editing Achievement:
    - Ability to effectively use CRISPR systems to combine varying amounts of genetic material from two species at the germline level.
  - Biomolecular Engineering Achievement:
    - Ability to grow germ cells for multiple species *in vitro* for easy gene editing.
    - Isolation and/or sequencing of genomic DNA from old, poorly preserved samples.
    - Develop CRISPR cassette delivery system to be able to effectively and efficiently edit embryos.
  - Host Engineering Achievement:
    - Develop viable hosts capable of successful breeding/pollinating, with life spans similar to original species.
  - Data Integration, Modeling, and Automation Achievement:
    - Better predictive long-term environmental and population models incorporating climate change data and engineered organism behavior/spread.

- Science/Engineering Aim 3: Develop high-resolution biosensors for environmental monitoring and detection.
  - Engineering Biology Objective 1: Develop better environmental biosensors for the detection of chemicals, radiation, temperature, pH, and water quality.
    - Gene Editing, Synthesis, and Assembly Achievement:
      - Large scale synthesis of biosensor variants, across kingdoms (from bacteria/archaea to plants).
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
      - Improved biosensor/genetic circuit designs for a multitude of sensor inputs.
      - Develop RNA or protein-based biosensors to detect and measure metabolites of interest.
    - Host and Consortia Engineering Achievement:
      - Create organisms that can act as multiplexing sensors capable of analyzing multiple environmental cues and providing measurable responses or combination of responses that may be deconvoluted to determine stimuli.
    - Data Integration, Modeling, and Automation Achievement:
      - Build more extensive and fully-sequenced metagenomics databases/libraries to enable searches for diverse functionalities across multiple gene clusters.

- Better enable real-time data feeds.

Societal Challenge 4: Enable sustainable, more environmentally-friendly materials and infrastructure development.

- Science/Engineering Aim 1: Enable sustainable production of environmentally-friendly consumable materials.
  - Engineering Biology Objective 1: Engineer organisms to produce durable, biodegradable materials (including plastics).
    - Gene Editing, Synthesis, and Assembly Achievement:
      - Synthesis of gene clusters to produce and export materials.
      - Implement necessary genetic mutations to improve cell fitness during production.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
      - Engineer biological polymers with the desired properties (such as strength, flexibility, and permeability).
      - Engineer channels that secrete biopolymers to make purification easier and reduce potential toxic side-effects on host organism.
      - Develop recyclable and degradable plastics from monomers that can be readily produced from renewable resources using engineered organisms.
    - Host and Consortia Engineering Achievement:
      - Microbes that produce relevant monomers.
      - Microbes that polymerize monomers into desired polymer morphology (such as branching and chain length).
    - Data Integration, Modeling, and Automation Achievement:
      - Design and model genetic programs for pathways and engineered metabolisms in select organisms.
- Science/Engineering Aim 2: Enable the production of sustainable construction materials.
  - Engineering Biology Objective 1: Development and production of fast-growing trees that produce stronger, fire-resistant wood.
    - Gene Editing, Synthesis, and Assembly Achievement:
      - Vectors and transformation systems for a desirable trees and vegetation.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
      - Enzymes and pathways that catalyze increased tannin deposition in plant tissue.
    - Host and Consortia Engineering Achievement:
      - Enable potential controlled propagation of engineered trees and vegetation.
    - Data Integration, Modeling, and Automation Achievement:
      - Identify genetic programs to improve resistance to fire through increased water uptake, tannin deposition, and taproot depth (similar to strategies employed by coastal redwoods).

        - Ability to predict and address factors limiting the scale-up of fast-growing, fire-resistant wood.
- Science/Engineering Aim 3: Improved management of the built environment using bio-designed and -enabled tools and technologies, including replacement of non-natural infrastructure with engineered organisms. (Engagement Example: By leveraging engineered enzymes during biofuel fermentation, byproducts of this process can be re-used to create more environmentally-friendly construction materials. See https://poet.com/asphalt for this example in action.)
    - Engineering Biology Objective 1: Engineer and produce organisms to replace or augment energy-consuming infrastructure or reduce energy consumption.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Enable tissue-specific gene expression in higher-order eukaryotes (such as in the twigs of deciduous trees or in needles of conifers).
            - Introduce luciferase and luciferin into a variety of trees (such as to create glowing plants and trees for natural, carbon-negative lighting in urban areas).
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Produce and advance engineered enzymes and other biomolecules for energy-consuming processes.
            - Enable incorporation of photosynthetic pathways into infrastructure biomaterials (such as for the production of carbon-negative bio-concrete).
        - Host and Consortia Engineering Achievement:
            - Engineer tree branches and leaves to maximize canopy area to increase summer shading.
        - Data Integration, Modeling, and Automation Achievement:
            - Use modeling to design a tree with the optimum canopy area to increase summer shading.
            - Ability to design low-load (cyclic) bioluminescence pathways that generate luciferin/luciferase at pre-specified times and/or in response to external stimuli.

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