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# U.S. DEPARTMENT OF ENERGY
# OFFICE OF ENERGY EFFICIENCY AND RENEWABLE ENERGY
# NEPA DETERMINATION

**RECIPIENT:** Lygos, Inc. **STATE:** CA

**PROJECT TITLE:** Accelerating engineered microbe optimization through machine learning and multiomics datasets

| Funding Opportunity Announcement Number | Procurement Instrument Number | NEPA Control Number | CID Number |
|---|---|---|---|
| DE-FOA-0001916 | DE-EE0008489 | GFO-0008489-001 | GO8489 |

**Based on my review of the information concerning the proposed action, as NEPA Compliance Officer (authorized under DOE Policy 451.1), I have made the following determination:**

**CX, EA, EIS APPENDIX AND NUMBER:**

Description:

| | |
|---|---|
| **A9 Information gathering, analysis, and dissemination** | Information gathering (including, but not limited to, literature surveys, inventories, site visits, and audits), data analysis (including, but not limited to, computer modeling), document preparation (including, but not limited to, conceptual design, feasibility studies, and analytical energy supply and demand studies), and information dissemination (including, but not limited to, document publication and distribution, and classroom training and informational programs), but not including site characterization or environmental monitoring. (See also B3.1 of appendix B to this subpart.) |
| **B3.6 Small-scale research and development, laboratory operations, and pilot projects** | Siting, construction, modification, operation, and decommissioning of facilities for smallscale research and development projects; conventional laboratory operations (such as preparation of chemical standards and sample analysis); and small-scale pilot projects (generally less than 2 years) frequently conducted to verify a concept before demonstration actions, provided that construction or modification would be within or contiguous to a previously disturbed or developed area (where active utilities and currently used roads are readily accessible). Not included in this category are demonstration actions, meaning actions that are undertaken at a scale to show whether a technology would be viable on a larger scale and suitable for commercial deployment. |

Rationale for determination:

The U.S. Department of Energy (DOE) is proposing to provide federal funding to Lygos, Inc. to design, develop, and test an aerobic, low pH yeast process for production of malonic acid. Strains of P. kudriavzevii would be optimized to improve malonic acid production through multiple fermentation cycles, in which experimental data would be used to inform machine learning algorithms designed to select for optimal strain characteristics. The project would be completed over three Budget Periods (BPs), with a Go/No-Go Decision Point in between each BP.

Proposed project activities for BP1 would focus on establishing baseline malonic acid fermentation performance data. Baseline fermentations would be performed and data would be analyzed. BP2 activities would include fermentation optimization through Design-Built-Test-Learn (DBTL) cycles, development of high-throughput multi-omics (e.g. metabolomics, transcriptomics, and proteomics) protocols, machine learning analysis of strain performance, metabolic network model development, training machine learning algorithms, and implementation of high-throughput test cycle fermentations. BP3 activities would consist of experimental validation of fermentation performance, metabolic network model refinement, machine learning optimization and high-throughput fermentations, incorporating machine learning and data on past fermentations.

All activities would be performed by Lygos and its project partners, Lawrence Berkeley National Laboratory (LBNL – Berkeley, CA), Sandia National Laboratory (SNL – Albuquerque, NM), and Pacific Northwest National Laboratory (PNNL – Richland, WA), at existing, purpose-built laboratory facilities. Activities to be performed by Lygos, at its laboratory facilities in Berkeley, CA, would include fermentations of engineered yeast microbes and analysis of resulting data. PNNL would perform global proteomic analysis on samples provided by Lygos and metabolic network modeling. LBNL would perform both targeted proteomic analysis and metabolomic analysis on fermentation samples. SNL would perform transcriptomic analysis on samples. All work activities would be completed indoors, in laboratory settings. Lygos and its project partners regularly conduct work similar in nature to that included as part of this project. No change in the use, mission or operation of existing facilities would be required. Likewise, no additional permits, licenses or authorizations would be needed.

Project work would include the use and handling of cellulosic glucose, yeast strains, organic solvents and industrial chemicals. All material handling would be performed in laboratory environments adequately equipped for the tasks included in the scope of work. Any risks associated with material handling would be mitigated through adherence to established health and safety policies and procedures. Protocols would include training of laboratory personnel, monitoring and oversight, engineering controls, and the use of personal protective equipment; including, fume hoods, safety glasses, laboratory coats, and chemically resistant gloves. Lygos and its project partners would also adhere to hazardous waste removal protocols that comply with government standards. All project activities would be performed in accordance with Federal, state, and local health, safety, and environmental regulations.

Genetic modifications would be performed as part of this project. National Institutes of Health (NIH) guidelines for handling BSL-1 microbes would be followed when performing laboratory activities involving genetically engineered microbes. All genetically engineered microbes would undergo heat or chemical sterilization prior to disposal in accordance with NIH guidelines.

Any work proposed to be conducted at a DOE laboratory may be subject to additional NEPA review by the cognizant DOE NEPA Compliance Officer for the specific DOE laboratory prior to initiating such work. Further, any work conducted at a DOE laboratory must meet the laboratory's health and safety requirements.

**NEPA PROVISION**

DOE has made a final NEPA determination.

Include the following condition in the financial assisstance agreement:

Any work proposed to be conducted at a federal facility may be subject to additional NEPA review by the cognizant federal official and must meet the applicable health and safety requirements of the facility.

Notes:

Bioenergy Technologies Office
This NEPA determination requires a tailored NEPA provision.
Review completed by Jonathan Hartman, 11/12/2018

**FOR CATEGORICAL EXCLUSION DETERMINATIONS**

The proposed action (or the part of the proposal defined in the Rationale above) fits within a class of actions that is listed in Appendix A or B to 10 CFR Part 1021, Subpart D. To fit within the classes of actions listed in 10 CFR Part 1021, Subpart D, Appendix B, a proposal must be one that would not: (1) threaten a violation of applicable statutory, regulatory, or permit requirements for environment, safety, and health, or similar requirements of DOE or Executive Orders; (2) require siting and construction or major expansion of waste storage, disposal, recovery, or treatment facilities (including incinerators), but the proposal may include categorically excluded waste storage, disposal, recovery, or treatment actions or facilities; (3) disturb hazardous substances, pollutants, contaminants, or CERCLA-excluded petroleum and natural gas products that preexist in the environment such that there would be uncontrolled or unpermitted releases; (4) have the potential to cause significant impacts on environmentally sensitive resources, including, but not limited to, those listed in paragraph B(4) of 10 CFR Part 1021, Subpart D, Appendix B; (5) involve genetically engineered organisms, synthetic biology, governmentally designated noxious weeds, or invasive species, unless the proposed activity would be contained or confined in a manner designed and operated to prevent unauthorized release into the environment and conducted in accordance with applicable requirements, such as those listed in paragraph B(5) of 10 CFR Part 1021, Subpart D, Appendix B.
There are no extraordinary circumstances related to the proposed action that may affect the significance of the environmental effects of the proposal.
The proposed action has not been segmented to meet the definition of a categorical exclusion. This proposal is not connected to other actions with potentially significant impacts (40 CFR 1508.25(a)(1)), is not related to other actions with individually insignificant but cumulatively significant impacts (40 CFR 1508.27(b)(7)), and is not precluded by 40 CFR 1506.1 or 10 CFR 1021.211 concerning limitations on actions during preparation of an environmental impact statement.
The proposed action is categorically excluded from further NEPA review.

**SIGNATURE OF THIS MEMORANDUM CONSTITUTES A RECORD OF THIS DECISION.**

NEPA Compliance Officer Signature: Electronically Signed By: Casey Strickland Date: 11/14/2018

NEPA Compliance Officer

**FIELD OFFICE MANAGER DETERMINATION**

- [x] Field Office Manager review not required
- [ ] Field Office Manager review required

**BASED ON MY REVIEW I CONCUR WITH THE DETERMINATION OF THE NCO :**

Field Office Manager's Signature: _____ Date: _____

Field Office Manager