

GRANTS/CONTINGENT AWARD REQUEST



To: Grants and Loans Office

Date: 4 / 23 / 2012

Project Manager: Akasha Kaur Khalsa

Phone Number: (916) 657-4854 ext.

Office: Special Projects Office

Division: Fuels and Transportation Division

MS- 23

Project Title: Fermentable Sugars for Ethanol from Microalgal Biomass Project

Type of Request: (check one)

**New Agreement:** (include items A-F from below) Agreement Number: Assigned by the G&L Office

Program: Alternate and Renewable Fuel Vehicle Technology (ARFVT)

Solicitation Name and/or Number: PON-11-601

Legal Name of Recipient: Kent BioEnergy Corporation

Recipient's Full Mailing Address: 11125 Flintkote Avenue  
San Diego, California 92121

Recipient's Project Officer: James Levin & Jim Carlberg Phone Number: (858) 452-5765 ext.

Agreement Start Date: 7 / 1 / 2012 Agreement End Date: 12 / 31 / 2014

**Amendment:** (Check all that apply) Agreement Number: ARV-11-020

Term Extension – New End Date:     /    /    

Work Statement Revision (include Item A from below)

Budget Revision (include Item B from below)

Change of Scope (include Items A – F as applicable from below)

Other: (Specify)



ITEMS TO ATTACH WITH REQUEST:

- A. Work Statement
- B. Budget
- C. Recipient Resolution, if applicable. (Resolution may be requested in Special Conditions if not currently available.)
- D. Special Conditions, if applicable.
- E. CEQA Compliance Form
- F. Other Documents as applicable
  - Copy of Score Sheets
  - Copy of Pre-Award Correspondence
  - Copy of All Other Relevant Documents

California Environmental Quality Act (CEQA)

CEQA finds, based on recipient's documentation in compliance with CEQA:

Project exempt: Section 15301 NOE filed:     /    /    

Environmental Document prepared: Type NOD filed:     /    /    

Other:     

CEC has made CEQA finding described in CEC-280, attached

Funding Information:

\*Source #1: ARFVTP Amount: \$ 1,496,426.00 Statute: 10 FY: 10/11 Budget List #: 601.118C

\*Source #2:      Amount: \$      Statute:      FY:      Budget List #:     

\*Source #3:      Amount: \$ 0.00 Statute:      FY:      Budget List #:     

If federally funded, specify federal agreement number:     

\* Source Examples include ERPA, PIER-E, PIER-NG, FED, GRDA, ARFVT, OTHER.

Business Meeting Approval: (refer to Business Meeting Schedule)

Proposed Business Meeting Date: 6 / 13 / 2012  Consent  Discussion

Business Meeting Participant: Akasha Kaur Khalsa Time Needed: 5 minutes

Agenda Notice Statement: (state purpose in layperson terms)

Possible approval of a  Grant /  Contingent Award to...

Kent BioEnergy Corporation for \$1,496,426 for the Fermentable Sugars for Ethanol from Microalgal Biomass Project to develop processes and assess commercial fuel production feasibility. The project will develop methods of cell lysis and separation of the carbohydrate component; invent bio-engineered sugar releasing enzymes; and produce lab-scale quantities of fermentable sugars and ethanol. (AB 118 funding)

Akasha Kaur Khalsa 4-24-12 Marcia Smith 4/25/12 Pat Perry 4/25/12

Project Manager Date Office Manager Date Deputy Director Date

Marcia Smith 4/24/12

RESOLUTION NO: [XX-XXXX-XX]

STATE OF CALIFORNIA

STATE ENERGY RESOURCES  
CONSERVATION AND DEVELOPMENT COMMISSION

RESOLUTION REGARDING: GRANT AWARD  
TO  
KENT BIOENERGY CORPORATION  
UNDER PON-11-601

**RESOLVED**, that the State Energy Resources Conservation and Development Commission (Energy Commission) approves **Grant Award # ARV-XX-XXX** with Kent BioEnergy Corporation (Recipient), for **\$1,496,426.00**, to research and produce fermentable sugars for ethanol production from microalgal biomass.

**WHEREAS**, the Energy Commission finds that the activities funded by this grant are a “project” under the California Environmental Quality Act (CEQA) and categorically exempt from further environmental review pursuant to the “existing facility” and “information collection” exemptions under CEQA Guidelines, §§ 15301 and 15306, respectively.

**FURTHER BE IT RESOLVED**, that this document authorizes the Executive Director or his/her designee to execute the same on behalf of the Energy Commission.

CERTIFICATION

The undersigned Secretariat to the Commission does hereby certify that the foregoing is a full, true, and correct copy of a RESOLUTION duly and regularly adopted at a meeting of the California Energy Commission held on [DATE]:

AYE: [List Commissioners]

NAY: [List Commissioners]

ABSENT: [List Commissioners]

ABSTAIN: [List Commissioners]

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Harriet Kallemeyn,  
Secretariat

**EXHIBIT A  
SCOPE OF WORK**

**TECHNICAL TASK LIST**

<b>Task #</b>	<b>CPR</b>	<b>Task Name</b>
1		Agreement Management
2		Biomass Production
3		Cell Lysis
4		Carbohydrate Isolation and Characterization
5		Software Development
6	X	Enzyme Engineering
7		Ethanol Test Fermentations
8		Data Collection and Analysis

**KEY NAME LIST**

<b>Task #</b>	<b>Key Personnel</b>	<b>Key Subcontractor(s)</b>	<b>Key Partner(s)</b>
1	Jim Carlberg – KBE Barry Olafson – Protabit	Protabit	
2	Jim Carlberg – KBE Greg Schwartz – KBE James Levin – KBE Christian Henrich – KBE Junior Valencia – KBE		
3	David Dodds – D&A LLC Christian Henrich – KBE Tim Wells – KBE		
4	David Dodds – D&A LLC Barry Toyonaga – KBE		
5	Barry Olafson – Protabit Benjamin Allen – Protabit Michael Dudek – Protabit Amos Anderson – Protabit Athanasios Dousis – Protabit	Protabit	
6	Stephen Mayo – Caltech Toni Lee – Caltech Jan Kostecki – Caltech Alex Nisthal – Caltech Rhonda DiGiusto – Caltech Barry Olafson – Protabit Heidi Privett – Protabit	Stephen Mayo (Caltech) Protabit	

Task #	Key Personnel	Key Subcontractor(s)	Key Partner(s)
	Athanasios Dousis – Protabit Marie Ary – Protabit		
7	David Dodds – D&A LLC		
8	David Dodds – D&A LLC KBE Team Protabit Team Caltech Team Monserate Team	Protabit Stephen Mayo (Caltech)	

## GLOSSARY

*Specific terms and acronyms used throughout this work scope are defined as follows:*

Acronym	Definition
ARFVT	Alternative and Renewable Fuel and Vehicle Technology
BG	Beta-glucosidase
BX	Beta-xylosidase
CAM	Commission Agreement Manager
CBH	Cellobiohydrolase
CI	Carbon Intensity
CPD	Computational protein design
CPR	Critical Project Review
EG	Endoglucanase
EX	Endoxylanase
GHG	Greenhouse Gas
HTS	High-throughput screen
KBE	Kent BioEnergy Corporation
LCFS	Low Carbon Fuel Standard
Recipient	Kent BioEnergy Corporation

## Background

Assembly Bill 118 (Núñez, Chapter 750, Statutes of 2007), created the Alternative and Renewable Fuel and Vehicle Technology Program (ARFVT Program). The statute, subsequently amended by AB 109 (Núñez, Chapter 313, Statutes of 2008), authorizes the Energy Commission to develop and deploy alternative and renewable fuels and advanced transportation technologies to help attain the state’s climate change policies. The Energy Commission has an annual program budget of approximately \$100 million and provides financial support for projects that:

- Develop and improve alternative and renewable low-carbon fuels;
- Optimize alternative and renewable fuels for existing and developing engine technologies;

- Produce alternative and renewable low-carbon fuels in California;
- Decrease, on a full fuel cycle basis, the overall impact and carbon footprint of alternative and renewable fuels and increase sustainability;
- Expand fuel infrastructure, fueling stations, and equipment;
- Improve light-, medium-, and heavy-duty vehicle technologies;
- Retrofit medium- and heavy-duty on-road and non-road vehicle fleets;
- Expand infrastructure connected with existing fleets, public transit, and transportation corridors; and
- Establish workforce training programs, conduct public education and promotion, and create technology centers.

The California Energy Commission issued solicitation PON-11-601 for Advanced Biofuel Production to provide funding opportunities under the ARFVT Program for the development of new, California-based biofuel production facilities that can sustainably produce low carbon transportation, for new, low carbon facilities, or for projects that lower the carbon intensity of fuels produced at existing facilities. To be eligible for funding under PON-11-601, the projects must also be consistent with the Energy Commission's ARFVT Program Investment Plan updated annually.

In response to PON-11-601, Kent BioEnergy Corporation (Recipient) submitted application # 13, which was proposed for funding in the Energy Commission's Notice of Proposed Awards, Round 1 on March 23, 2012, and is incorporated by reference to this Agreement in its entirety.

### **Problem Statement**

Ethanol as a fuel additive is widely accepted as a beneficial strategy for the displacement of fossil-based fuels because current ethanol production is generated from renewable plant sources, which assimilate CO<sub>2</sub> from the atmosphere during feedstock growth. A further refinement of ethanol feedstock is the pursuit of cellulosic ethanol sources since they offer an immediate solution to the food vs. fuel dilemma presented by first generation corn starch derived ethanol. Using carbohydrate sources from non-edible feedstock is also a viable option, and using total carbohydrate (cellulosic and starch) from algal biomass offers great promise.

A number of factors are impeding the rapid and wide-scale use of cellulosic feedstock to date. Most, if not all, of them lead to economic concerns including, but not limited to biomass production cost, transportation of biomass to refinery, preparation of cellulosic feedstock from biomass, inefficiencies of enzymatic conversion to fermentable sugars, and enzyme costs.

### **Goals of the Agreement**

The goal of this project is to develop novel, scalable, and economic processes to produce fermentable sugar feedstock from microalgae. The overall technical goals of the proposal can be broken down into two categories: (a) the development of economic methods for isolating the carbohydrate fraction from microalgae, and (b) the

identification and optimization of sugar-releasing enzymes required to generate fermentable sugars from algae-derived carbohydrates.

### **Objectives of the Agreement**

The objectives of this proposal are to maximize and quantify the release of fermentable sugars from microalgae cellulose followed by determination of total ethanol yield in standard ethanol fermentation assays.

## **TASK 1 ADMINISTRATION**

### **Task 1.1 Attend Kick-off Meeting**

The goal of Task 1.1 is to establish the lines of communication and procedures for implementing this Agreement.

#### **The Recipient shall:**

- Attend a “Kick-Off” meeting with the Commission Agreement Manager (CAM), the Grants Officer, and a representative of the Accounting Office. The Recipient shall bring its Project Manager, Agreement Administrator, Accounting Officer, and others designated by the CAM to this meeting.
- The administrative and technical aspects of this Agreement will be discussed at the meeting. Prior to the kick-off meeting, the CAM will provide an agenda to all potential meeting participants. The administrative portion of the meeting shall include, but not be limited to, discussion of the following:
  - Terms and Conditions of the Agreement
  - Critical Project Review (Task 1.2)
  - Match fund documentation (Task 1.6).
  - Permit documentation required (Task 1.7)
  - Subcontracts needed to carry out project (Task 1.8)The technical portion of the meeting shall include, but not be limited to, discussion of the following:
  - The CAM’s expectations for accomplishing tasks described in the Scope of Work
  - Schedule of Products
  - Monthly Progress Reports (Task 1.4)
  - Technical Products (Product Guidelines located in Section 5 of the Terms and Conditions)
  - Final Report (Task 1.5)The CAM shall designate the date and location of this meeting.

#### **Recipient Products:**

- Updated Schedule of Products
- Updated List of Match Funds
- Updated List of Permits

**CAM Product:**

- Kick-Off Meeting Agenda

**Task 1.2 Critical Project Review (CPR) Meetings**

The goal of Task 1.2 is to determine if the project should continue to receive Energy Commission funding to complete this Agreement and to identify any needed modifications to the tasks, products, schedule or budget.

A Critical Project Review (CPR) provides the opportunity for frank discussions between the Energy Commission and the Recipient. CPRs generally take place at key, predetermined points in the Agreement, as determined by the CAM and as shown in the Technical Task List above. However, the CAM may schedule additional CPRs as necessary, and any additional costs will be borne by the Recipient.

Participants include the CAM and the Recipient and may include the Commission Grants Officer, the Fuels and Transportation Division (FTD) team lead, other Energy Commission staff and Management as well as other individuals selected by the CAM to provide support to the Energy Commission.

**The CAM shall:**

- Determine the location, date, and time of each CPR meeting with the Recipient. These meetings generally take place at the Energy Commission, but they may take place at another location.
- Send the Recipient the agenda and a list of expected participants in advance of each CPR. If applicable, the agenda shall include a discussion on both match funding and permits.
- Conduct and make a record of each CPR meeting. One of the outcomes of this meeting will be a schedule for providing the written determination described below.
- Determine whether to continue the project, and if continuing, whether or not modifications are needed to the tasks, schedule, products, and/or budget for the remainder of the Agreement. Modifications to the Agreement may require a formal amendment (please see the Terms and Conditions, Section 8). If the CAM concludes that satisfactory progress is not being made, this conclusion will be referred to the Transportation Committee for its concurrence.
- Provide the Recipient with a written determination in accordance with the schedule. The written determination may include a requirement for the Recipient to revise one or more product(s) that were included in the CPR.

**The Recipient shall:**

- Prepare a CPR Report for each CPR that discusses the progress of the Agreement toward achieving its goals and objectives. This report shall include recommendations and conclusions regarding continued work on the projects. This report shall be submitted along with any other products identified in this scope of work. The Recipient shall submit these

- documents to the CAM and any other designated reviewers at least 15 working days in advance of each CPR meeting.
- Present the required information at each CPR meeting and participate in a discussion about the Agreement.

**CAM Products:**

- Agenda and a list of expected participants
- Schedule for written determination
- Written determination

**Recipient Product:**

- CPR Report(s)

**Task 1.3 Final Meeting**

The goal of Task 1.3 is to closeout this Agreement.

**The Recipient shall:**

- Meet with Energy Commission staff to present the findings, conclusions, and recommendations. The final meeting must be completed during the closeout of this Agreement.

This meeting will be attended by, at a minimum, the Recipient, the Commission Grants Officer, and the CAM.

The technical and administrative aspects of Agreement closeout will be discussed at the meeting, which may be two separate meetings at the discretion of the CAM.

During the technical portion of the meeting the Recipient shall present an assessment of the degree to which project and task goals and objectives were achieved, findings, conclusions, recommended next steps (if any) for the Agreement, and recommendations for improvements. The CAM will determine the appropriate meeting participants.

The administrative portion of the meeting shall include a discussion with the CAM and the Grants Officer about the following Agreement closeout items:

- What to do with any equipment purchased with Energy Commission funds (Options)
- Energy Commission's request for specific "generated" data (not already provided in Agreement products)
- "Surviving" Agreement provisions
- Final invoicing and release of retention
- Prepare a schedule for completing the closeout activities for this Agreement.

**Products:**

- Written documentation of meeting agreements
- Schedule for completing closeout activities

**Task 1.4 Monthly Progress Reports**

The goal of Task 1.4 is to periodically verify that satisfactory and continued progress is made toward achieving the research objectives of this Agreement on time and within budget.

The objectives of this task are to summarize activities performed during the reporting period, to identify activities planned for the next reporting period, to identify issues that may affect performance and expenditures, and to form the basis for determining whether invoices are consistent with work performed.

**The Recipient shall:**

- Prepare a Monthly Progress Report which summarizes all Agreement activities conducted by the Recipient for the reporting period, including an assessment of the ability to complete the Agreement within the current budget and any anticipated cost overruns. Each progress report is due to the CAM within 10 days of the end of the reporting period. The recommended specifications for each progress report are contained in Section 6 of the Terms and Conditions of this Agreement.
- In the first Monthly Progress Report and first invoice, document and verify match expenditures and provide a synopsis of project progress, if match funds have been expended or if work funded with match share has occurred after the notice of proposed award but before execution of the grant agreement. If no match funds have been expended or if no work funded with match share has occurred before execution, then state this in the report. All pre-execution match expenditures must conform to the requirements in the Terms and Conditions of this Agreement.

**Product:**

- Monthly Progress Reports

**Task 1.5 Final Report**

The goal of Task 1.5 is to produce a Final Report which describes the project's success in achieving its goals and objectives, advancing science and technology, and providing energy-related and other benefits to California.

The objectives of the Final Report are to clearly and completely describe the project's purpose, approach, activities performed, results, and advancements in science and technology; to present a public assessment of the success of the project as measured by the degree to which goals and objectives were achieved; to make insightful observations based on results obtained; to draw conclusions; and to make recommendations for further projects and improvements to the ARFVT project management processes.

The Final Report shall be a public document. If the Recipient has obtained confidential status from the Energy Commission and will be preparing a confidential version of the Final Report as well, the Recipient shall perform the following activities for both the public and confidential versions of the Final Report.

**The Recipient shall:**

- Prepare an Outline of the Final Report.
- Prepare a Final Report following the approved outline and the latest version of the Final Report guidelines which will be provided by the CAM. The CAM shall provide written comments on the Draft Final Report within fifteen (15) working days of receipt. The Final Report must be completed at least 60 days before the end of the Agreement Term.
- Submit one bound copy of the Final Report with the final invoice.

**Products:**

- Draft Outline of the Final Report
- Final Outline of the Final Report
- Draft Final Report
- Final Report

**Task 1.6 Identify and Obtain Matching Funds**

The goal of Task 1.6 is to ensure that the match funds planned for this Agreement are obtained for and applied to this Agreement during the term of this Agreement.

The costs to obtain and document match fund commitments are not reimbursable through this Agreement. Although the Energy Commission budget for this task will be zero dollars, the Recipient may utilize match funds for this task. Match funds shall be spent concurrently or in advance of Energy Commission funds for each task during the term of this Agreement. Match funds must be identified in writing and the associated commitments obtained before the Recipient can incur any costs for which the Recipient will request reimbursement.

**The Recipient shall:**

- Prepare a letter documenting the match funding committed to this Agreement and submit it to the CAM at least 2 working days prior to the kick-off meeting. If no match funds were part of the proposal that led to the Energy Commission awarding this Agreement and none have been identified at the time this Agreement starts, then, state such in the letter. If match funds were a part of the proposal that led to the Energy Commission awarding this Agreement, then provide in the letter a list of the match funds that identifies the:
  - Amount of each cash match fund, its source, including a contact name, address and telephone number and the task(s) to which the match funds will be applied.

- Amount of each in-kind contribution, a description, documented market or book value, and its source, including a contact name, address and telephone number and the task(s) to which the match funds will be applied. If the in-kind contribution is equipment or other tangible or real property, the Recipient shall identify its owner and provide a contact name, address and telephone number, and the address where the property is located.
- Provide a copy of the letter of commitment from an authorized representative of each source of cash match funding or in-kind contributions that these funds or contributions have been secured. For match funds provided by a grant, a copy of the executed grant shall be submitted in place of a letter of commitment.
- Discuss match funds and the implications to the Agreement if they are reduced or not obtained as committed, at the kick-off meeting. If applicable, match funds will be included as a line item in the progress reports and will be a topic at CPR meetings.
- Provide the appropriate information to the CAM if during the course of the Agreement additional match funds are received.
- Notify the CAM within 10 days if during the course of the Agreement existing match funds are reduced. Reduction in match funds must be approved through a formal amendment to the Agreement and may trigger an additional CPR.

**Products:**

- A letter regarding match funds or stating that no match funds are provided
- Copy(ies) of each match fund commitment letter(s) (if applicable)
- Letter(s) for new match funds (if applicable)
- Letter that match funds were reduced (if applicable)

**Task 1.7 Identify and Obtain Required Permits**

The goal of Task 1.7 is to obtain all permits required for work completed under this Agreement in advance of the date they are needed to keep the Agreement schedule on track.

Permit costs and the expenses associated with obtaining permits are not reimbursable under this Agreement. Although the Energy Commission budget for this task will be zero dollars, the Recipient shall budget match funds for any expected expenditures associated with obtaining permits. Permits must be identified in writing and obtained before the Recipient can make any expenditure for which a permit is required.

**The Recipient shall:**

- Prepare a letter documenting the permits required to conduct this Agreement and submit it to the CAM at least 2 working days prior to the kick-off meeting. If there are no permits required at the start of this Agreement, then state such in the letter. If it is known at the beginning of the Agreement that permits will be required during the course of the Agreement, provide in the letter:

- A list of the permits that identifies the:
  - Type of permit
  - Name, address and telephone number of the permitting jurisdictions or lead agencies
- The schedule the Recipient will follow in applying for and obtaining these permits.
- Discuss the list of permits and the schedule for obtaining them at the kick-off meeting and develop a timetable for submitting the updated list, schedule and the copies of the permits. The implications to the Agreement if the permits are not obtained in a timely fashion or are denied will also be discussed. If applicable, permits will be included as a line item in the Progress Reports and will be a topic at CPR meetings.
- If during the course of the Agreement additional permits become necessary, provide the appropriate information on each permit and an updated schedule to the CAM.
- As permits are obtained, send a copy of each approved permit to the CAM.
- If during the course of the Agreement permits are not obtained on time or are denied, notify the CAM within 5 working days. Either of these events may trigger an additional CPR.

**Products:**

- Letter documenting the permits or stating that no permits are required
- A copy of each approved permit (if applicable)
- Updated list of permits as they change during the term of the Agreement (if applicable)
- Updated schedule for acquiring permits as changes occur during the term of the Agreement (if applicable)

**Task 1.8 Manage Subcontracts**

The goal of this task is to ensure quality products and to procure subcontracts required to carry out the tasks under this Agreement consistent with the terms and conditions of this Agreement and the Recipient's own procurement policies and procedures. It will also provide the Energy Commission an opportunity to review the subcontracts to ensure that the tasks are consistent with this Agreement, that the budgeted expenditures are reasonable and consistent with applicable cost principles.

**The Recipient shall:**

- Manage and coordinate subcontractor activities.
- Submit a draft of each subcontract required to conduct the work under this Agreement to the Commission Agreement Manager for review.
- Submit a final copy of the executed subcontract.
- If Recipient decides to add new subcontractors, it shall notify the Commission Agreement Manager.

**Products:**

- Draft subcontracts
- Final subcontracts

**Task 1.9 Test Plan Preparation**

The goal of Task 1.9 is to prepare the test plan for carbohydrate isolation and hydrolysis. The commercial implementation of the technology to be developed in this proposal is dependent upon the associated costs so the efficiency of each process step will be evaluated. The three critical processes are cell lysis, carbohydrate fractionation, and enzymatic hydrolysis of polysaccharides.

**The Recipient shall:**

- Prepare and submit the Carbohydrate Isolation and Hydrolysis Test Plan which will describe the research outlined in Tasks 2 through 7 in greater detail. This plan shall include, but is not limited to:
  - a description of the process to be tested;
  - the rationale for why the tests are required;
  - predicted performance based on calculations or other analyses;
  - test objectives and technical approach;
  - a test matrix showing the number of test conditions and replicated runs;
  - a description of the facilities, equipment, instrumentation required to conduct the tests;
  - a description of test procedures, including parameters to be controlled and how they will be controlled; parameters to be measured and instrumentation to measure them; calibration procedures to be used; recommended calibration interval; and maintenance of the test log;
  - a description of the data analysis procedures;
  - a description of quality assurance procedures;
  - contingency measures to be considered if the test objectives are not met
- Conduct research outlined in Tasks 2-7 in accordance with the Carbohydrate Isolation and Hydrolysis Test Plan.

**Products:**

- Carbohydrate Isolation and Hydrolysis Test Plan

**TECHNICAL TASKS**

**Task 2 Biomass Production**

The goal of Task 2 is to produce algal biomass using algae species that will be adaptable to large-scale outdoor pond cultivation and waste-derived nutrients. This biomass, also called feedstock, will be used for the process development described in

Task 2 Cell Lysis, Task 3 Fractionation, and Tasks 6.2 / 6.4 Carbohydrate Hydrolysis. Task 2 also includes participation in the algae industry's preeminent, national conference. A site visit by a subject matter expert on biomass characteristics will be organized.

**The Recipient shall:**

- Select algal strains suitable for outdoor cultivation.
- Cultivate strains under laboratory conditions monitoring various growth metrics.
- For select strains, vary cultivation conditions (light, temperature, salinity, and pH) to determine potential variation that may occur in algae based feedstock.
- Harvest biomass and generate algal biomass (1-10% solids).
- Record data on the algal biomass produced including quantity, cultivation conditions, and algae composition.
- Prepare and submit an Algal Biomass Production Summary Report that includes the methodologies employed and results obtained under this task.
- Obtain input and evaluation from algal biomass production expert on the effect of different waste streams on biomass quality and quantity at Recipient's facility.
- Participate in the Algal Biomass Summit (ABS) in September 2012. The ABS is the algae industry's premier national meeting and will facilitate discussions with other biomass producers.
- Explore biomass processing equipment options.

**Products:**

- Algal Biomass Production Summary Report

**Task 3 Cell Lysis**

The goal of Task 3 is to investigate different methods of inducing microalgae cell lysis in the feedstock. Methods will be evaluated based on their cost effectiveness and degree of cell wall disruption. This task will also include a meeting with a commercial producer of processing equipment and a site visit by Dr. Dodds, the process development expert.

**The Recipient shall:**

- Implement different methods of inducing microalgae cell lysis in accordance with the Carbohydrate Isolation and Hydrolysis Test Plan. .
- Obtain input from a process development expert to evaluate methods, equipment, and overall process efficiency for suitability for commercial production at scale.
- Develop and submit Cell Lysis protocol.
- Update the Cell Lysis protocol, if necessary.
- Evaluate sonication methods of lysis.
- Evaluate pressure differential methods of lysis.

- Evaluate mechanical disruption for lysis.
- Evaluate enzymatic methods of lysis.
- Evaluate industrial biomass processing machinery to obtain equipment price quotes for various methods of lysis .
- Prepare and submit a Cell Lysis Report on the various lysis methods tested and their efficiency at liberating carbohydrate relative to the cost of the process.

**Products:**

- Cell Lysis Report
- Cell Lysis Protocol

**Task 4 Cell Lysate Fractionation: Carbohydrate Isolation and Characterization**

The goals of Task 4 are to: (1) Develop processes to isolate the carbohydrate component of the total cell lysate. (2) Determine the carbohydrate, lipid and protein composition of the lysate. (3) Evaluate lipid and protein fractions for use as animal/fish feed additives and fertilizer. (4) Meet with various companies involved in relevant industries relating to commercial development of grant outcomes. Generating multiple profit streams from algal biomass is a key component to KBE's commercialization strategy.

**The Recipient shall:**

- Develop a fractionation protocol.
- Determine the physical characteristics of the lysate (pH, viscosity, adventitious proteolytic activity).
- Evaluate membrane filtration for lysate fractionation and carbohydrate separation.
- Evaluate chromatographic methods for fractionation and carbohydrate separation.
- Evaluate action of carbohydrate hydrolyzing enzymes on lysate prior to fractionation.
- Determine the most cost effective method to fractionate biomass.
- Track solubility and carbohydrate content of process streams.
- Track lipid and protein content of process streams to allow eventual use as animal feed.
- Update the fractionation protocol.
- Determine industrial carbohydrate processors' interest in purchasing algal-based carbohydrates, product standards, and pricing arrangements.
- Open communication with major industrial entities that refine, blend, and directly market E10 and E85 to investigate demand for product streams resulting from algal biomass.
- Explore options for lipid and protein process streams to be used as industrial chemical feedstock
- Prepare and submit a Cost Effectiveness of Lysate Fractionation Report on the most cost effective method to fractionate biomass that includes the

effectiveness of carbohydrate separation, methodologies employed, results obtained under this task, and the data generated.

- Prepare and submit a Carbohydrate Fraction Report on the composition of the fractions.
- Prepare and submit an Animal Feed/Fish Feed Additives and Fertilizers from Microalgae Report that evaluates lipid and protein fractions for use as animal/fish feed additives and fertilizer that includes the methodologies employed and results obtained under this task.

**Products:**

- Animal Feed/Fish Feed Additives and Fertilizers from Microalgae Report
- Cost Effectiveness of Lysate Fractionation Report
- Carbohydrate Fraction Report
- Fractionation Protocol

**Task 5 Software Development**

The goal of Task 5 is to enhance the Protabit enzyme engineering platform by developing new algorithms and refining the core tools based on experimental results.

**The Recipient shall:**

- Codify thermostability design features into an easy-to-use software application.
- Build/refine score functions to incorporate pH and salt effects.
- Refine software platform based on feedback from experiments.
- Review and discuss work performed quarterly, in face-to-face meetings, at the discretion of the Recipient.
- Prepare and submit a Software Upgrade Features and Improved Capabilities List of new features and improved capabilities.

**Products:**

- Software Upgrade Features and Improved Capabilities List

**Task 6 Enzyme Engineering**

**[A CPR will be held during this Task. See Task 1.2 for details.]**

**Task 6.1 Express Core Enzyme Set**

The goal of Task 6.1 is to express the core set of wild-type hydrolytic enzymes.

**The Recipient shall:**

- Obtain gene sequences for wild-type enzymes from GenBank, or similar source.
- Optimize genes for expression host (*P. pastoris* or *S. cerevisiae*) codon usage and add 6-histidine tag to the end of each gene.
- Synthesize genes (DNA 2.0).
- Incorporate genes into appropriate expression vectors (Invitrogen).

- Transform genes into expression host using commercial yeast transformation kits.
- Sequence genes.
- Verify individual sequences and adequate representation of members of combinatorial libraries.
- Express enzymes in high-throughput format.
- Analyze for purity using nickel-affinity chromatography.
- Prepare and submit a Core Enzymes and Expression Yields List that includes the results obtained under this sub-task.

**Products:**

- Core Enzymes and Expression Yields List

**Task 6.2 Determine Optimal Mixture of Core Set of Wild-Type Enzymes**

The goal of Task 6.2 is to determine the optimal mixture of enzymes needed for complete hydrolysis of microalgal cell wall carbohydrates.

**The Recipient shall:**

- Develop a high-throughput enzymatic activity assay for the processed (fractionated) feedstock. This will be a microplate assay using commercial colorimetric glucose/xylose assay kits and a robotic liquid handling system.
- Optimize the activity assay using a commercial hydrolytic enzyme preparation.
- Determine activity of commercial hydrolytic enzymes:
  - At vendor-recommended hydrolysis temperature (50°C) and pH (5)
  - At multiple elevated temperatures (50-80°C) and pH (4-8)
- Determine optimal mixture of core wild-type enzymes based on activity.
- Determine activity of optimal wild-type enzyme mixture at multiple elevated temperatures (50-80°C) and pH (4-8). Calculate T50 (incubation temperature at which the enzymes lose half of their activity).
- Prioritize core enzymes based on activity at initial and elevated temperatures, synergy with other enzymes, pH profile, importance in maximizing sugar yield, etc. Highest priority enzymes will be engineered first.
- Prepare and submit an Optimal Mixture of Core Set of Wild-Type Enzymes Report that details the methodologies employed and results obtained under this sub-task.
- Prepare and submit Activity Profiles of Wild-type and Commercial Enzymes, that details the results obtained under this sub-task.
- Prepare and submit a Prioritized Enzymes to be Engineered List that details the results obtained under this sub-task.

**Products:**

- Optimal Mixture of Core Set of Wild-Type Enzymes Report
- Activity Profiles of Wild-type and Commercial Enzymes
- Prioritized Enzymes to be Engineered List

**Task 6.3 Engineer Enzymes in Core Set**

The goal of Task 6.3 is to engineer enzymes in core set to maintain hydrolytic activity at elevated temperatures and target pH. Enzymes will be engineered in order of priority as determined in Task 6.2.

**The Recipient shall:**

- Perform computational designs and generate libraries of variant sequences.
- Express variant libraries consistent with Task 6.1.
- Screen variant libraries for activity at elevated temperature and/or target pH; screening will always be done in the context of the set of core enzymes from Task 6.1.
- Refine thermostable designs to optimize/regain activity at target pH.
- Incorporate activity assay data into new computational designs.
- Repeat above subtasks until variants with desired activity at elevated temperatures and/or target pH are obtained.
- Identify best variants.
- Review and discuss work performed quarterly, in face-to-face meetings, at the discretion of the Recipient.
- Prepare and submit an Enzyme Engineering Progress Report that details the methodologies employed and results obtained under this sub-task.

**Products:**

- Enzyme Engineering Progress Report

**Task 6.4 Determine and Characterize Optimal Mixture of Best Variants**

The goal of Task 6.4 is to determine the optimal mixture of the highest-performing engineered core enzymes and characterize this set for comparison to the wild-type core enzymes and commercial enzyme preparations.

**The Recipient shall:**

- Determine optimal mixture of engineered core enzymes based on activity under desired hydrolysis conditions (pH and temperature).
- Determine activity of optimal engineered enzyme mixture at multiple elevated temperatures (50-80°C) and pH (4-8). Calculate T50 (consistent with Task 6.2).
- Determine crystal structures of selected variants (non-hits as well as best hits) if deemed useful for improved understanding and feedback for future designs.

- Prepare and submit an Engineered Enzymes Activity Profiles Report that details the methodologies employed and results obtained under this sub-task.

**Products:**

- Engineered Enzymes Activity Profiles Report

**Task 7 Ethanol Test Fermentations**

The primary goal of Task 7 is to achieve validation of generated carbohydrates as fermentation feedstock for ethanol production (lab scale). A secondary goal is to estimate ethanol and sugar conversion costs based on fermentable sugar yields, enzyme loading, and ethanol conversion. This task also includes a meeting with the ethanol test fermentation sub-contractor and conferences relating to the ethanol industry.

**The Recipient shall:**

- Determine glucose/xylose yield as a function of hydrolysis time and enzyme loading.
- Determine composition of hydrolysate (i.e., process stream generated by enzymatic hydrolysis).
- Perform standard yeast fermentations of the hydrolysate at laboratory (shake-flask) scale using standard yeast strain(s).
- Calculate total ethanol production based on total biomass.
- Calculate total ethanol production based on total cell carbohydrate.
- Calculate total ethanol production based on fractionated carbohydrate.
- Evaluate the carbohydrate fraction for use as fuel feedstock.
- Update fermentation protocol with advice from the minor sub-contractor NREL.
- Research the most current technical and business issues facing the advanced biofuels market at the National Advanced Biofuels Conference and submit results to the Commission Agreement Manager through one regular monthly progress report and the final report.
- Determine the requirements for industrial carbohydrate feedstocks, ethanol production, and the fuel additives markets and submit results to the Commission Agreement Manager through one regular monthly progress report and the final report.
- Prepare and submit an Ethanol Test Fermentations Report that details the methodologies employed and results obtained under this task.

**Products:**

- Ethanol Test Fermentations Report

## **Task 8 Data Collection and Analysis**

The goal of Task 8 is to collect and analyze operational data to determine the economic viability and environmental impact of the project. Final analysis of all project data must be included in the Final Report.

### **The Recipient Shall:**

- Collect data from biomass conversion system to include:
  - Efficiency of conversion of feedstock.
  - Biofuel production rate.
  - Quantity of fuel produced.
- Estimate gasoline and/or petroleum-based diesel fuel that will be displaced annually.
- Explain how the project will reduce criteria air pollutants and air toxics and reduce or avoid multimedia environmental impact, and lead to a decrease, on a life-cycle basis, in emissions of water pollutants or any other substances known to damage human health or the environment.
- Explain how the project incorporated and achieved the sustainability goals.
- Provide a quantified estimate of the project's carbon intensity values for life-cycle scale greenhouse gas emissions.
- Quantify any water efficiency and water use reduction measures used in the project including, but not limited to, the use of recycled or reclaimed water and the reduction or elimination of point and nonpoint source wastewater discharge.
- Describe any potential use of renewable energy or cogeneration in the project.
- Collect and provide data on expected job creation, economic development, and increased state revenue.
- Compare any project performance and expectations provided in the proposal to Energy Commission with actual project performance and accomplishments.
- Describe how the project supports new technology advancement for vehicles, vessels, engines, and other equipment, and promote the deployment of such technologies in the marketplace. To the extent possible, describe how the project provided a measurable transition from the nearly exclusive use of petroleum fuels to a diverse portfolio of viable alternative fuels that meets California's petroleum reduction and alternative fuel use goals.
- Describe how the project demonstrated the cost-effectiveness of the proposed technology in achieving greenhouse gas emissions reduction.
- Provide additional data that may be requested by the Energy Commission during the term of this Agreement, as is reasonably available.

### **Products:**

- None. Information shall be included in the Final Report.

Notice of Exemption

To: Office of Planning and Research
P.O. Box 3044, Room 212
Sacramento, CA 95812-3044

From: (Public Agency) \_\_\_\_\_

County Clerk
County of \_\_\_\_\_

(Address)

Project Title: \_\_\_\_\_

Project Location - Specific:

Project Location - City: \_\_\_\_\_ Project Location - County: \_\_\_\_\_

Description of Nature, Purpose and Beneficiaries of Project:

Name of Public Agency Approving Project: \_\_\_\_\_

Name of Person or Agency Carrying Out Project: \_\_\_\_\_

Exempt Status: (check one)

- Ministerial (Sec. 21080(b)(1); 15268);
Declared Emergency (Sec. 21080(b)(3); 15269(a));
Emergency Project (Sec. 21080(b)(4); 15269(b)(c));
Categorical Exemption. State type and section number:
Statutory Exemptions. State code number:

Reasons why project is exempt:

Lead Agency
Contact Person: \_\_\_\_\_ Area Code/Telephone/Extension: \_\_\_\_\_

If filed by applicant:

- 1. Attach certified document of exemption finding.
2. Has a Notice of Exemption been filed by the public agency approving the project? Yes No

Signature: \_\_\_\_\_ Date: \_\_\_\_\_ Title: \_\_\_\_\_

- Signed by Lead Agency
Signed by Applicant
Date received for filing at OPR: \_\_\_\_\_