CRADA Final Report **Date: June 22, 2016**

PI __Taek Soon Lee_______

CRADA No. _CRADA FP00001042 (AWD0000350)____
LBNL Report Number _1005778

1. **Parties:** Afingen, Inc.

2. **Title of the Project:** "Engineering Robust Yeasts for Biorefinery Applications"

3. **Summary of the specific research and project accomplishments**:
Isoprene is highly-valued terpene based-chemical feedstock and can be derived from either petroleum or from fermentation of plant biomass. This project enabled more efficient isoprene fermentation using renewable resources and at yields that can compete economically with non-renewable sources. This Phase I project applied a novel synthetic biology approach, the Artificial Positive Feedback Loop (APFL) technology, to improve production yields of isoprene.

4. **Deliverables**:

Deliverable Achieved	Party (LBNL, Participant, Both)	Delivered to Other Party?
Plasmids	Participant	No
E.coli with plasmid	Participant	No
S. cerevisiae with	Participant	No

5. **Identify publications or presentations at conferences directly related to the CRADA?**
Presented at *Seed central*, a public-private partnership from UC Davis for communication & research collaboration between the industry and UC Davis in order to bring science to market faster.

6. **List of Subject Inventions and software developed under the CRADA:**
N/A

7. **A final abstract suitable for public release:**
Many isoprenoid derivatives can be made by heterogonous synthetic pathways in engineered microorganisms, but at large scale production, the low toxic tolerance and growth defects of conventional host organisms have resulted in low yield of target molecules. The eukaryotic organism Saccharomyces cerevisiae have been extensively studied as favored hosts for metabolic engineering in research projects due to easy availability of classical genetic approaches and accessibility of modern molecular biological tools. In this project, we applied

novel synthetic biology approach (APFL) to improve production yields of isoprene, the fundamental building block of isoprenoids, in Saccharomyces cerevisiae laboratory strain.

8. Benefits to DOE, LBNL, Participant and/or the U.S. economy.

U.S. energy and economic security depend on efficient chemical production, but renewable sources of hydrocarbon feedstocks are needed to transition to environmental sustainability. Demand for renewable hydrocarbon chemicals is rapidly growing and is expected to continue rising for several decades. However, current biorefineries face cost competitiveness challenges due to inadequate yield, need to pre-process feedstocks, lack of robust fermentation hosts, and limited ability to produce a range of products to maximize income compared to petrochemical counterparts. In the chemical industry, isoprenoid are highly valued as chemical feedstocks for a broad range of industrial products. Current isoprenoid supply largely depends on petroleum. A sustainable source of isoprenoid would be environmentally desirable, but current biorefineries are unable to compete. Synthetic biology, a recent industrial biotechnology approach, promises large-scale and geographically flexible supplies of the terpenes via fermentation of plant sugars and polysaccharides waste. Afingen, Inc., University of California at Berkeley and Lawrence Berkeley National Laboratory developed a suite of synthetic biology tools, collectively called the Artificial Positive Feedback Loop (APFL), which enabled the engineering of microorganisms to amplify specific metabolic pathways without deleterious effects to the overall health of the organism. Consequently, a low-cost renewable isoprenoid production platform will be applicable to a wide range of isoprenoid products including flavors and fragrances, food ingredients, fine chemicals, crop protection products and pharmaceuticals.

9. Financial Contributions to the CRADA:

DOE Funding to LBNL	$ 0
Participant Funding to LBNL	$ 54,109
Participant In-Kind Contribution Value	$ 74,008
Total of all Contributions	$ 128,117

June 24, 2016

SUMMARY REPORT

Isoprene is highly-valued terpene feedstock and can be derived from either petroleum or from fermentation of plant biomass. This project enabled more efficient isoprene fermentation using renewable resources and at yields that can compete economically with non-renewable sources. This Phase I project applied a novel synthetic biology approach, the Artificial Positive Feedback Loop (APFL) technology, to improve production yields of isoprene.

JBEI Project Team: Taek Soon Lee, Director of Metabolic Engineering (PI)

Afingen Project Team: Brad Niles, Ruthie Chow, and Ai Oikawa

Objective: Afingen planned to demonstrate that the Artificial Positive Feedback Loop (APFL) technology, developed at Lawrence Berkeley National Laboratory to improve production of high value compounds in yeast, can be used to improve isoprene production in various yeast strains. In this project, Afingen aimed at [1] Developing APFL constructs for yeast transformation and transform yeast with them, and [2] Validation of the APFL for isoprene production.

Results Summary:

1. **Design of the DNA constructs:** We designed DNA constructs to a biosynthesis of isoprene to the ergosterol pathway and generated several APFLs to boost precursor production from the ergosterol biosynthesis.
2. **Validation of APFL for isoprene production in laboratory strain *S. cerevisiae* BY4742:** We validated the benefit of the APFL technology in a non-optimized yeast strain for the production of isoprene. A plasmid harboring the isoprene synthase was integrated in the genome of S. cerevisiae BY4742 strain at the rDNA locus using high-efficiency LiAc transformation. Isolated and confirmed transformants was retransformed with different APFLs constructs. Several independent colonies were isolated and genomic integration

were verified by PCR. The selected transformants were analyzed for isoprene production. The analysis were performed with the support of the Metabolic Engineering group led by Taek Soon Lee from the Joint BioEnergy Institute as they perform routinely this type of analysis.

3. **Rapid technology translation to other yeast strains:** We planned to transfer of different APFL and pathways used for the production of isoprene and its producers to highly-divergent other fungi strains. We made some knock out transformatns for future APFL translation to the species by an established transformation method.

4. **Conclusion:** Many isoprenoid derivatives can be made by heterogonous synthetic pathways in engineered microorganisms, but at large scale production, the low toxic tolerance and growth defects of conventional host organisms have resulted in low yield of target molecules. Yeasts are the industrial workhorses of the bioethanol industry and have proven their economic feasibility in large-scale facilities converting biomass into ethanol. However, fermentation production of biofuel molecules of higher energy density has been problematic due to the low toxicity tolerance of the typical yeast strains currently in use. This SBIR Phase I project applied Lawrence Berkeley National Laboratory (LBNL)'s novel synthetic biology approach (APFL) to dramatically improve production yields of valuable high energy density compounds. The broader testing of the APFL technology in new constructs of engineered yeast strains enabled a more benign and more efficient method to amplify production of high-value target compounds. The target advanced biofuel compounds in this project was isoprene which are produced from precursors derived from the well-known yeast isoprenoid-ergosterol biosynthesis pathway. The isoprene have great potential in renewable energy since they can be rapidly converted into biofuels after a simple biofuel conversion. Renewable biofuels and biofuel precursor products are fast growing markets and demand will keep rising for decades. Upon further demonstration of the APFL technology in engineered yeast strains, a large variety of yeasts will be able to be engineered to biologically synthesize biofuels and specialty biofuel precursor compounds.