

Synthetic Biology Internal Review Process and Biosecurity Screening

March 24, 2015
Nathan J. Hillson



SynBio Internal Review Committee



Purpose

- Review all Synthetic Biology user proposals
- Primary focus on environmental, biocontainment, biosafety, and biosecurity aspects
- Consider ethical, legal, and societal aspects

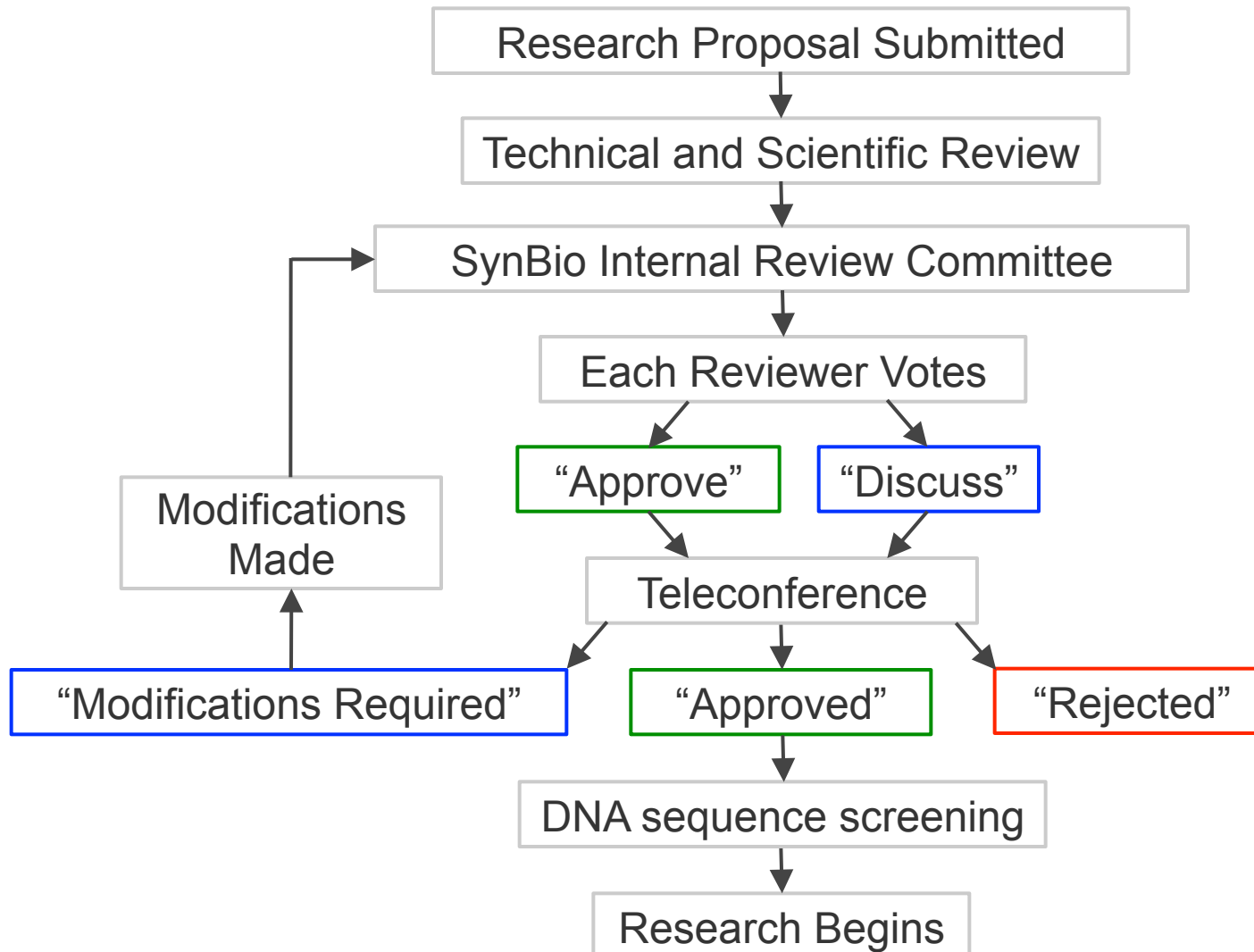
Composition

- Berkeley Lab staff
- External experts
- Members of the public (in the future)

This committee is one of the firsts of its kind

- The JGI is providing leadership
- Other institutions could adopt this successful process
- Review process software will facilitate replication

SynBio Internal Review Process



Investigator Guidelines



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Synthetic Biology Internal Review Process – Investigator Guidelines

This web page provides guidance for Investigators as they prepare their JGI DNA synthesis proposal submissions in anticipation of the Synthetic Biology Internal Review process.

Background

Synthetic biology has the potential to accelerate science and bolster economic growth. However, like any new technology, synthetic biology could be misapplied or result in unintended consequences. Legitimate concerns have been raised over the intentional use of synthetic biology approaches to engineer pathogenic organisms and the accidental environmental release of genetically engineered organisms. Scientists pursuing synthetic biology research must diligently consider issues such as these.

Overview of the JGI Synthetic Biology Internal Review Process

The JGI Synthetic Biology Internal Review process seeks to assess, beyond technical and scientific merit, the broader aspects (e.g., environmental, biosafety, biosecurity) of the research proposals associated with the JGI's DNA synthesis program. The purpose of this internal review process is two-fold: 1) to assess the broader aspects of the research, request proposal modifications if issues of concern are not sufficiently addressed in the proposal, reject research proposals where issues of

Web-based Review System

Welcome Nathan Hillson

SBIRC #:2

(BRC)WIP#1552: Mining Evolutionary Space for Improved Biomass Deconstruction Enzymes

Submitted by [redacted] on 2013.12.17

The cost of enzymes for converting lignocellulosic biomass into fermentable sugars is a major obstacle to the economical development of second generation biofuels. The most promising enzyme mixtures are derived from fungi, such as *Trichoderma reesei*, *Aspergillus oryzae*, and *Myceliophthora thermophila*. In these mixtures, a dominant enzyme is cellobiohydrolase 1 (CBH1, also known as Cel7A). CBH1 is an exo-acting enzyme that sequentially removes cellobiose units from the reducing end of crystalline cellulose. It is the rate-limiting enzyme for the conversion of cellulose to glucose. As such, it is the single most important enzyme in the lignocellulosic ethanol pipeline. Little work has been done to improve CBH1 in heterologous systems such as *E. coli*, *S. cerevisiae*, or *P. pastoris*, due to heavy O- and N-glycosylation and 10 disulfide bonds. In this project, we propose to express CBH1 in *T. reesei* itself. Our lab has extensive experience working with filamentous fungi including transformation-mediated gene knockouts and over-expression in *T. reesei*. Glycosyl hydrolase (GH) family 7, to which CBH1 belongs, is found only in eukaryotes. In the last few years, the genomes of many fungi and other lower eukaryotes that produce CBH1 orthologs have been sequenced. We propose to exploit these new DNA resources and our experience with fungal molecular genetics to survey the evolutionary space of CBH1, with the goal of identifying a CBH1 that is superior to TrCBH1. Furthermore, by measuring the kinetic constants, pH profiles, and temperature optima of the different enzymes, our results will contribute to our knowledge of the evolutionary potential of this important family of enzymes. The genes will need to be synthesized because obtaining cDNA versions of the genes for expression is not practical. Many of the genomes that we will mine are obligate pathogens or otherwise difficult or impossible to culture.

Download

Comments:

General [1]

BioSafety [2]

BioSecurity [0]

Submitted by [redacted] on 2014.01.14

I agree that it is safer to use specific synthetic sequences related to toxicity, pathogenicity or virulence than the biology is possibly in favor of the proposal. However, as the sequences used are related to pathogenicity, I agree that we will mine are obligate pathogens or otherwise difficult or impossible to culture.

Submitted by [redacted] on 2014.01.14

The proposal does not associate any biosafety case.

Synthetic Biology Internal Review

Proposal SBIRC#: 2

Submitted by [redacted] on 2013.12.17

Final Determination:

Approved on 2014.02.04

Title:

(BRC)WIP#1552: Mining Evolutionary Space for Improved Biomass Deconstruction Enzymes

Abstract:

The cost of enzymes for converting lignocellulosic biomass into fermentable sugars is a major obstacle to the economical development of second generation biofuels. The most promising enzyme mixtures are derived from fungi, such as *Trichoderma reesei*, *Aspergillus oryzae*, and *Myceliophthora thermophila*. In these mixtures, a dominant enzyme is cellobiohydrolase 1 (CBH1, also known as Cel7A). CBH1 is an exo-acting enzyme that sequentially removes cellobiose units from the reducing end of crystalline cellulose. It is the rate-limiting enzyme for the conversion of cellulose to glucose. As such, it is the single most important enzyme in the lignocellulosic ethanol pipeline. Little work has been done to improve CBH1. The main impediment to the lack of progress is the difficulty of expressing CBH1 in heterologous systems such as *E. coli*, *S. cerevisiae*, or *P. pastoris*, due to heavy O- and N-glycosylation and 10 disulfide bonds. In this project, we propose to express CBH1 in *T. reesei* itself. Our lab has extensive experience working with filamentous fungi including transformation-mediated gene knockouts and over-expression in *T. reesei*. Glycosyl hydrolase (GH) family 7, to which CBH1 belongs, is found only in eukaryotes. In the last few years, the genomes of many fungi and other lower eukaryotes that produce CBH1 orthologs have been sequenced. We propose to exploit these new DNA resources and our experience with fungal molecular genetics to survey the evolutionary space of CBH1, with the goal of identifying a CBH1 that is superior to TrCBH1. Furthermore, by measuring the kinetic constants, pH profiles, and temperature optima of the different enzymes, our results will contribute to our knowledge of the evolutionary potential of this important family of enzymes. The genes will need to be synthesized because obtaining cDNA versions of the genes for expression is not practical. Many of the genomes that we will mine are obligate pathogens or otherwise difficult or impossible to culture.

Full proposal attached at end of report

Full proposal attached at end of report

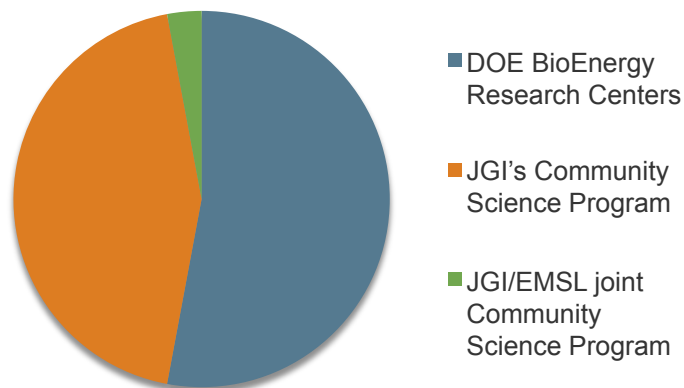
Decision Notes

Review Committee Decision Notes:

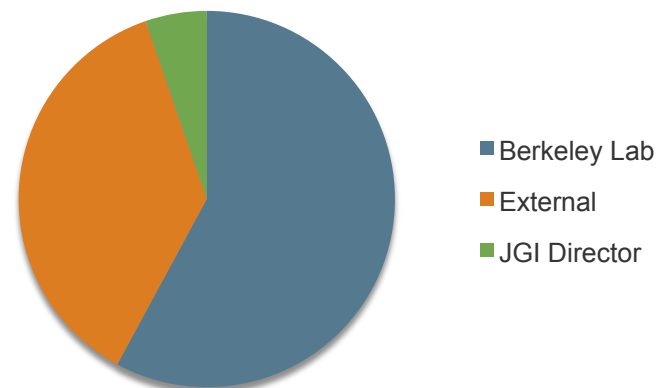
It wasn't clear that the proposal was sufficiently considering potential adverse environmental consequences given the scale of how much protein would need to be produced for a viable biofuels industry. The final vote would still have been approve after discussion.

SynBio Internal Review Stats

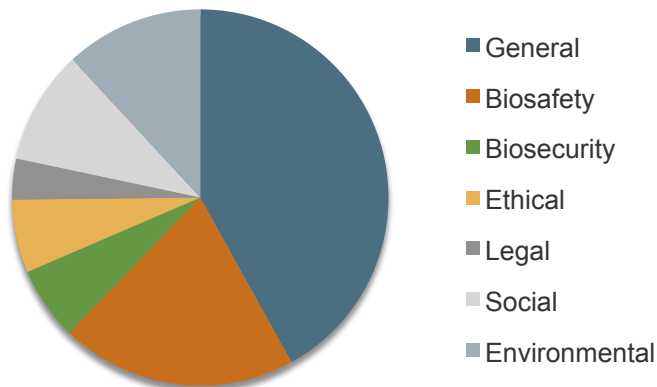
34 Proposals Reviewed



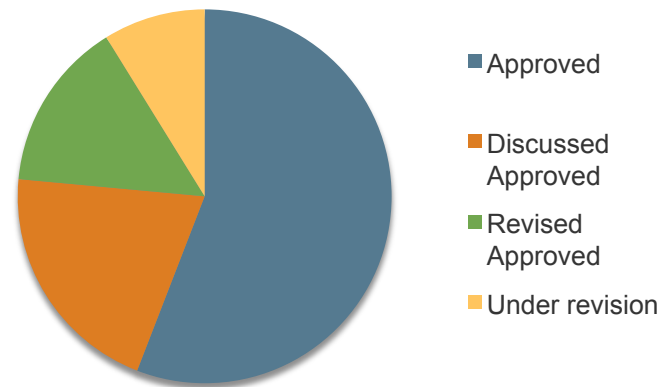
19 Reviewers



143 Reviewer Comments



Proposal Review Decisions



Biosecurity screening



International Gene Synthesis Consortium

- Harmonized screening protocol

User screening

- “Black lists” from U.S. Commerce, State, and Treasury Depts.
- Visual Compliance (VC) software for restricted party screening

Sequence screening

- “Sequences of concern”
- Select Agents and Toxins; Commerce and EU control lists
- New software (GenoGuard-inspired) for sequence screening



Sequence screening example

Sequence screening results for project: Batch086

1. ❌ Batch86_p001 - Status: FAILED, Flag: RED - [More...](#)
2. ❌ Batch86_p002 - Status: FAILED, Flag: RED - [More...](#)
3. ❌ Batch86_p003 - Status: FAILED, Flag: RED - [More...](#)
4. ❌ Batch86_p004 - Status: FAILED, Flag: RED - [More...](#)
5. ❌ Batch86_p005 - Status: FAILED, Flag: RED - [More...](#)
6. ❌ Batch86_p006 - Status: FAILED, Flag: RED - [More...](#)
7. ❌ Batch86_p007 - Status: FAILED, Flag: RED - [More...](#)
8. ❌ Batch86_p008 - Status: FAILED, Flag: RED - [More...](#)
9. ❌ Batch86_p009 - Status: FAILED, Flag: RED - [More...](#)
10. ❌ Batch86_p010 - Status: FAILED, Flag: RED - [More...](#)
11. ❌ Batch86_p011 - Status: FAILED, Flag: RED - [More...](#)
12. ❌ Batch86_p012 - Status: FAILED, Flag: RED - [More...](#)



Color Coded Icon Legend

- ✔ **Passed Screening:** None of the alignments to the sequence were a "hit" (i.e. matched a blacklist item)
- ✔ **Passed Screening:** There were alignments to the sequence that were a "hit", but none of them were the "Best Match" for all 200bp windows
- ❌ **Failed Screening:** There were alignments to the sequence that were a "hit", and at least one of them was of the "Best Match" for a 200bp windows, where there was also a non-hit "Best Match", that was not directly comparable (i.e one was a DNA alignment, and the other was a AA alignment). The "hit" corresponded to an item on the *Select Agents and Toxins* list.
- ❌ **Failed Screening:** There were alignments to the sequence that were a "hit", and at least one of them was of the "Best Match" for a 200bp windows, The "hit" corresponded to an item on the *Select Agents and Toxins* list.
- ✔ **Export Controlled:** Is the same as ❌ except and the blacklist item is on **NOT** on the *Select Agents and Toxins* list, thus it is subject to Export Control only