Massachusetts Institute of Technology Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight

Biological Research Registration Form

Instructions:
This form is available at: http://ehs.mit.edu/site/content/biosafety-resources-and-links
Please download and save this form to your computer. When completing this document please retain the format as nearly as possible and answer questions thoroughly. Complete the appropriate sections as outlined below. **All information in this form is considered confidential.**

Please indicate all relevant biosafety levels and research descriptors that describe your research.

Biosafety Level ☒BL1 ☐BL2 ☐BL2+ ☐BL3

Research Descriptors		X	rDNA/sNA	X	Biological Agents BL1		Other		Human embryonic stem cells		Induced pluripotent stem cells
	Plants	x	Pathogens		Human Materials		Viral Vectors	X	Nanotechnology		Toxin Use

Required Information. Every Principal Investigator must complete the following:
• Section 1 (General Information)
• Section 2 (Laboratory Information)
• Section 3 (Research Description)
• Section 11 (Dual Use Assessment)
• Section 12 (Laboratory Safe Practices and Procedures)
• Section 13 (Certification and Signatures).

Research Specific Sections. Please complete the following sections if they are applicable to the research described here. **Please indicate either that the section has been completed or is not applicable (N/A).**		Was section completed?
Section 4	Teaching Laboratory/Biomaker space Information	☐ Completed ☒ N/A
Section 5	Use of Recombinant and Synthetic Nucleic Acid	☒ Completed ☐ N/A
Section 6	Biological Agent Use	☒ Completed ☐ N/A
Section 7	Use of Human Source Materials	☐ Completed ☒ N/A
Section 8	Use of Human Embryonic Stem (hES) Cells or Induced Pluripotent Stem (iPS) Cells	☐ Completed ☒ N/A
Section 9	Occupational Health Assessment, Medical Surveillance and Monitoring	☐ Completed ☒ N/A
Section 10	Use of Toxins	☐ Completed ☒ N/A

When you have completed the form, please email the completed form to your BSP contact or to BSP@mit.edu. Print and sign Section 13, the Certification and Signature page and mail this page to Biosafety Program, N52-496.

Section 1. General Information (required):

Current Registration number: 796	Title of Registration: **Synthetic Biology for Development of Biomaterials**	
Principal Investigator **Timothy Lu**	Departmental Affiliation/DLC: **EECS/RLE/BE/SBC**	
PI email address: **timlu@mit.edu**	PI office phone: 715-4808	PI email address: **timlu@mit.edu**
Lab Research Contact: Ky Lowenhaupt	Lab Administrative Contact: Rashmi Karki	
Lab Research Contact email address: kytsing@mit.edu	Lab Administrative Contact email address: rkarki@mit.edu	
Lab Research Contact phone: 4-8149	Lab Administrative Contact phone: 4-6492	

Section 2. Laboratory Information (required):

a. List ALL Laboratories/Facilities where research is to be conducted and the corresponding biosafety level: include cold/warm rooms, equipment rooms as appropriate. Please indicate room(s) where biosafety cabinets (BSC) are located. Please include the location(s) of the autoclave used for laboratory waste sterilization prior to disposal. (The box is expandable.)

		Check box if applicable					
Room Number	Biosafety Level	BSCs in room	Warm/ Cold Room	Equipment Room	Human Materials	hES/iPS cells used	Autoclave location
NE47-017B	BL1	1 BSC					Biowaste boxes
NE47-019	BL1						Biowaste boxes
NE47-209 – shared lab	BL2	1 BSC					Biowaste boxes
NE47-217 – shared tissue culture room	BL2	5 BSCs			X		Biowaste boxes
NE47-235	BL2						Biowaste boxes
NE47-265	BL2						Biowaste boxes
NE47-290C – shared cold room	BL2		Cold Room				Biowaste boxes
NE47-290D – shared autoclave/dish washing room	BL2			X			Biowaste boxes
NE47-335B	BL2						Biowaste boxes
NE47-335C	BL2						Biowaste boxes
36-797	BL2						Biowaste boxes
36-799 – autoclave/dish washing room	BL2			X			Biowaste boxes
36-781	BL2						Biowaste boxes
36-789	BL2						Biowaste boxes

b. Please list or attach a list of all laboratory personnel working on this Registration at MIT, to include faculty, technical staff, graduate students, UROPS, etc. (The box is expandable.)

Laboratory Personnel			Research Materials Used (place an X in the appropriate box)			Training Completed (Enter most recent date of training)	
Name	Kerberos	UROP (Y/N)	Uses BL1, BL2 material	Uses BL2+, BL3 material	Uses human material	General Biosafety training (260c)	Bloodborne Pathogens training
Cao, JiCong	jicong	N	X	NA	X	03/09/16	03/07/17
Chang, Cheng	Cchang1	N	X	N/A	X	04/24/15	03/07/17
Chen, Ying-Chou	Yjoechen	N	X	N/A		03/09/16	03/07/17
Chen, Willam	wcwchen	N	X	N/A		03/09/16	03/07/17

Citorik, Robert	Rcitorik	N	X	N/A	X	03/09/16	03/07/17
Da Luz Areosa Cleto, Sara	scleto	N	X	N/A		03/09/16	03/07/17
Farzadfard, Fahim	Ffard	N	X	N/A		03/09/16	03/07/17
De la Fuentes Nunez, Cesar	cfuente	N	X	N/A		03/09/16	03/07/17
Higashikuni, Yasutomi	tommyh	N	X	N/A	X	03/09/16	03/07/17
Harrison, Christina	tia7	N	X	N/A	X	06//02/14	03/07/17
Jerger, Logan	ljerger	N	X	N/A		07/14/16	03/07/17
Jung, Giyoung	giyoung	N	X	N/A		03/09/16	03/07/17
Jusiak, Barbara	jusiak	N	X	N/A	X	03/09/16	03/07/17
Lemire, Sebastien	Sele	N	X	N/A		03/09/16	03/07/17
Lowenhaupt, Ky	kytsing	N	X	N/A	X	03/09/16	03/07/17
Lu, Timothy	Timlu	N	X	N/A	X	03/09/16	03/07/17
Mimee, Mark	Mmimee	N	X	N/A	X	03/09/16	03/07/17
Mueller, Isaak	imueller	N	X	N/A		03/09/16	03/07/17
Nissim, Lior	Liorni	N	X	N/A	X	03/09/16	03/07/17
Nissim, Adina	anissim	N	X	N/A	X	03/09/16	03/07/17
Park, Heechul	heechul	N	X	N/A		03/09/16	03/07/17
Pery, Erez	erezpery	N	X	N/A	X	03/09/16	03/07/17
Sothiselvam, Shanmugapriya	Priya89	N	X	N/A		03/09/16	03/07/17
Sun, Qing	sunqing	N	X	N/A		03/09/16	03/07/17
Tang, Tzu-Chieh	tctang	N	X	N/A		03/09/16	03/07/17
Tham, Eleonore	etham	N	X	N/A		03/09/16	03/07/17
Wu, Ming-Ru	mingru	N	X	N/A	X	03/09/16	03/07/17
Yehl, Kevin	kyehl	N	X	N/A		03/09/16	03/07/17

Section 3. Research Description (required):

a. In lay terms, briefly describe your research as you would to a friend who is not a scientist.

The Synthetic Biology Group is focused on advancing fundamental designs and applications for synthetic biology. Using principles inspired by electrical engineering and computer science, we are developing new techniques for constructing, probing, modulating, and modeling engineered biological circuits. Our current application areas include infectious diseases, amyloid-associated conditions, and nanotechnology.

Many natural biological systems—such as biofilms, shells and skeletal tissues—are able to assemble multifunctional and environmentally responsive multiscale assemblies of living and non-living components. By using inducible genetic circuits and cellular communication circuits

to regulate *Escherichia coli* curli amyloid production, we show that *E. coli* cells can organize self-assembling amyloid fibrils across multiple length scales, producing amyloid-based materials that are either externally controllable or undergo autonomous patterning. We also interfaced curli fibrils with inorganic materials, such as gold nanoparticles (AuNPs) and quantum dots (QDs), and used these capabilities to create an environmentally responsive biofilm-based electrical switch, produce gold nanowires and nanorods, co-localize AuNPs with CdTe/CdS QDs to modulate QD fluorescence lifetimes, and nucleate the formation of fluorescent ZnS QDs. This work lays a foundation for synthesizing, patterning, and controlling functional composite materials with engineered cells. [*Nature Materials* 2014]

b. Outline the overall goal(s) of the research in the space below. Give enough information to assure that the purpose of the experiments and the techniques used are clear. Please use reasonably non-technical terms.

1: Synthetic biology strategies for the development of novel biomaterials

1.1) Engineering bacteria to control production of materials for both biological and inorganic structures

Diverse biological materials, ranging from cellulose to mussel glue, show useful properties that are hard to reproduce through chemistry and, thanks to the programmability of living organisms, are relatively easy to use as templates for hybrid living/non-living materials. Bacteria form adherent conglomerates called biofilms, creating strong, self-repairing, chemically resistant materials. A major component of the *E. coli* biofilm is curli, a long flexible proteaneceous surface appendage formed of polymers of a single protein, CsgA. We have engineered CsgA for geographic patterning of biofilm formation and functionalization with non-natural activities (Chen, A. Y. et al. (2014) Synthesis and patterning of tunable multiscale materials with engineered cells. Nat. Mater. 13, 515-523.). We are constantly exploring new uses for this living material.

- We are building genetic frameworks for light control of biofilm patterning in *E.coli* in collaboration with the Voigt lab at MIT. Different wavelengths of light trigger the expression of different versions of CsgA with different properties. A polyhistidine tag allows chelation of metals; SpyTag reacts with SpyCatcher to covalently link CsgA to fusion proteins. Biofilms can be created with precisely controlled areas of differing function with applications in bioremediation and bio-catalysis.
- Single-walled carbon nanotubes (SWNTs) are used in electronic applications because they allow high electron mobility. They also display near infrared (IR) light emission which renders them very desirable for biological imaging applications. Additionally, they can be easily functionalized with small peptides and proteins. Fused with SpyCatcher, they can be tethered to engineered *E. coli* biofilms and we are exploring the bioelectronics and imaging applications of such conjugates.
- Engineered filamentous bacteriophages such as M13 can biomineralize metal ions and precipitate them out of solution. Curli fibers can also be reprogrammed to bind metal ions and sequester them. Screens of random mutations in the M13 coat proteins P8 and P9 and of CsgA will be screened for improved binding to heavy metals. A matrix including both curli and M13 proteins will form a biofilter. We aim to combine

this biofilter with traditional activated charcoal to make efficient, inexpensive, and portable water filter membranes that can remove a wide range of heavy metal ions.

- Many organisms living underwater produce extremely strong adhesives based on assembled amyloids. Despite recent advances, our understanding of the molecular design, self-assembly, and structure-function relationships of these natural amyloid fibers remains limited. Thus, designing biomimetic amyloid-based adhesives remains challenging. We have reported strong and multi-functional underwater adhesives obtained from fusing mussel foot proteins (Mfps) of Mytilus galloprovincialis with CsgA proteins, the major subunit of *Escherichia coli* amyloid curli fibers. These hybrid molecular materials hierarchically self-assemble into higher-order structures, in which, according to molecular dynamics simulations, disordered adhesive Mfp domains are exposed on the exterior of amyloid cores formed by CsgA. Our fibers have an show underwater adhesion 1.5 times greater than any other reported bio-inspired and bio-derived protein-based underwater adhesives. We are continuing to modify the make-up of these fibers in order to further improve on their quality and to investigate their properties.

- Biofilms come in many varieties and while that formed by *E. coli* is largely proteaneceous, other organisms incorporate very different molecules in their biofilms leading to materials with radically different physico-chemical properties that we are also trying to harness. One project is to use *Gluconacetobacter xylinus* and yeasts (*S. cerevisiae, P. pastoris, Z. bisporus, B. bruxellensis*) to make functionalized cellulose membranes. To this end, we are identifying genes that make up the cellulose synthetic pathway from fungi or other microorganisms including Aspergillus, and introducing them into *Gluconacetobacter* or yeasts. By controlling the expression of these enzymes, we hope to tune the mechanical properties of the cellulose membrane. Because the tools for engineering *Gluconacetobacter* do not currently exist, the first goal is to modify tools for genetic engineering laboratory strains and assess their utility in *Gluconacetobacter.*

  Another group of candidates for functional living materials production is mushrooms such as *Ganoderma lucidum, T. versicolor, A. bisporus, P. ostreatus, P. microspora*). n the past decade, several groups have reported on the successful introduction and expression of foreign genes in higher fungi, however, these studies were mostly carried out by using random insertion-based techniques including restriction enzyme mediated integration (REMI) and Agrobacterium *tumefaciens* mediated transformation (ATMT). Thus, a precise and robust editing system is needed for site-specific manipulation of G. *lucidum* genome. Leveraging recent advances in CRISPR/Cas-based genome editing and the complete genome sequence of G. *lucidum* published in 2012, we will construct a built-in CRISPR/Cas system to engineer G. *lucidum* in a systematic fashion. To begin with, a cas9 gene will be integrated into a monokaryon G. lucidum strain using conventional transformation techniques. This cas9 gene will be control by a G. lucidum GPD promoter that allows constitutive expression under normal physiological conditions. Meanwhile, a single guide RNA (sgRNA) targeting user-defined DNA sequences and a repair template reporter gene (e.g. GFP) will be introduced to G.

lucidum on a plasmid. The efficiency of GFP knock-in and its expression level will be analyzed using flow cytometry.

Personnel contributing to this project are Eleonore Tham, Heechul Park, Tzu-Chieh Tang, Kevin Yehl, Qing Sun, and Tim Lu.

1.2) Encapsulation of bacteria in hydrogel compartments for biocontainment

Biosafety has become a major challenge for the transition of synthetic biology tools from the laboratory to the field. Genetic and chemical containment strategies have been implemented to restrict the growth and replication of genetically modified organisms but no robust physical containment has been proposed. We are developing a hydrogel-based encapsulation technique by leveraging a tough biocompatible shell and genetically recoded organisms to achieve unprecedented containment performance. This approach enables convenient sample retrieval, controlled lifespan, and protection against environmental insults. We demonstrate that the encapsulated cells can sense, respond, and record environmental inputs and use safe laboratory *E. coli* K-12 strains for our initial demonstrations.

Personnel contributing to this project are Eleonore Tham, Tzu-Chieh Tang, Heechul Park and Tim Lu.

Section 4. Teaching Laboratory / Biomaker space Information: Check box if not applicable ☒

a. Please complete the bulleted points below for a Teaching Lab.

Teaching Lab: ☐

- Number of students in the class:
- Category of students/participants (e.g. high school, undergraduate, graduate student):
- Student-Instructor Ratio:
- Experience level of course Teaching Assistants:
- Describe how any biological materials will be handled and who will handle the biological materials:

b. Please complete the bulleted points below for a Biomaker Space.

Biomaker Space: ☐

- Number of user in the space:
- Minimum experience of the participants (undergraduate, graduate student):
- Average Participant/Supervisor Ratio:
- Experience level of Supervisors:
- Estimated Core hours of operation:
- Briefly describe the training process and how proficiency will be verified for users; include any equipment specific training that will be given and by whom:
- Describe how access to the Biomaker space is granted to new users:
- List the equipment available in the Biomaker Space for use in biological experiments:
- Describe how inventory of biological material will be kept and the process for new material to be added to the Biomaker Space:

Section 5. Use of Recombinant or Synthetic Nucleic Acid: Check box if not applicable ☐ (Please complete this section if you use or generate recombinant microorganisms, cells, animals, plants, etc.)

Please indicate the Section of the NIH Recombinant DNA Guidelines that applies to this research. If you have questions Biosafety Staff will be glad to assist you. Note that the City of Cambridge requests that we register all recombinant work even if it is exempt under section III-F or Appendix C.

Section III-A ☐ **Section III-B** ☐ **Section III-C** ☐ **Section III-D** ☒ **Section III-E** ☒ **Section III-F** ☒

a. Source of Gene, Insert or Clone:

1. Specify DNA/RNA source (or probe), nature of insert, is a protein expressed, and percent of any viral genome in construct (for viral vectors see 5.b.2):

Insert	Cloned into	Under promoter control	Insert source	Nature of insert	Protein expressed?	% viral genome
Selection markers for E. coli including Bla, Kan, Zeocin resistance, etc	Plasmids described below	Native promoter	Plasmid collection	Antibiotic resistance	yes	<1%
Transcription factors including tetR, lacI, phlF,luxR, araC) †	Plasmids described below	Native promoter	Lab of T. Lu	Transcription factor	yes	<1%
Synthetic transcription factors (i.e. zinc fingers, TALEN) †	Plasmids described below	Synthetic	Synthetic, Lab of T Lu	Transcription factor	yes	<1%
gRNA	Plasmids described below					
Flurorescent proteins (i.e. GFP, RFP, BFP, YFP)	Plasmids described below	scTEF, AOX1, GAP, synthetic	Plasmid collection	Fluorescent proteins	yes	<1%
Enzymatic reporters such as lacZ, gusA	Plasmids described below	P_Y, pTraM, tac, T7, SP6, BAD, lac, OmpC, FixK2 and CGG	Lab of T Lu	Enzymes whos activity can be detected in a simple assay	yes	<1%
Recobminases and phage integrases (i.e. Top901 int, Bxb1 int, *λ int, cre* and *flp, recomvbinases from* Voigt library)	Plasmids described below	P_Y, pTraM, tac, T7, SP6, BAD, lac, OmpC, FixK2 and CGG	Lab of C Voigt, lab of T Lu	recombinase	yes	<1%
Biofilm structural proteins such as CsgA, mfp	pET-11d	T7	Lab strain Ecoli, cDNA libraries	Structural protein	yes	<1%

Light sensors, such as Cph8 (red), YF1/FixJ (blue) and CcaR/CcaS (green)	Plasmids described below	OmpC, FixK2, CGG, T3, K1F		Light sensors	yes	<1%
gRNAs	Plasmids described below	synthetic	synthetic	Guide RNAs	no	<1%
CRISPR Associated Proteins, such as Cas9 and Cas3 ##	Plasmids described below	P_Y, pTraM, tac, T7, SP6, BAD, lac, OmpC, FixK2 and CGG	Labs of T. Lu, C. Voigt	Endonuclease	yes	<1%
Enzymes in the cellulose synthetic pathway	Plasmids described below	P_Y, pTraM, tac, T7, SP6, BAD, lac, OmpC, FixK2 and CGG, synthetic	synthetic	Enzymes in metabolic pathway	yes	<1%

CRISPR/Cas9: We employ two systems, the streptococcus CRISPR/Cas9 system and the *E. coli* CRISPR/Cas3 developed by the Voigt lab. We have both natural endonuclease proficient and endonuclease deficient versions. **Cas9/dCas9**. Although the most commonly used CRISPR/cas system, from Streptococcus pyogenes, originates from a risk group 2 organism, in the experiments described herein this system has been cloned into only risk group 1 organisms, and no impact on biological safety is foreseen. All transformation uses chemoporation or electroporation, and no viral vectors are used.

† Transcription factors, both natural and synthetic will be used to modulate the expression of proteins tha comprise our biomaterials, and enzymes in biomaterial synthetic pathways. Regulation of the expression of transcription factors provides a more nuanced regulation of expression of the end product, and reflects the process seen in nature.

2. Do any sequences code for toxins? Yes ☐ No ☒; If yes, please specify.

3. Are any sequences from select agents and toxins? Yes ☐ No ☒; If yes, please specify.

4. Is the DNA source from a USDA-regulated plant, animal or insect? Yes ☐ No ☒; If the regulated organism is grown or stored at MIT, please include a copy of the USDA permit. (Link to USDA site: http://www.aphis.usda.gov/brs/index.html)

b. Vectors and Host Cells:

1. Identify cloning/expression/transfection vectors used, recipient bacterial strains, and recipient host cell lines (human, mouse, plant, etc.). Provide a restriction map of vector if it is new or not published. If commercially available vector or plasmid, please indicate vendor. Describe the location and type of promoters and other control sequences and percent of any viral genome in construct.

Vector name	Recipient Bacterial, yeast strain	Reporter gene?	% viral genes	promoters	Host cell
Shuttle vectors, i.e. pRS3xx/pRS4xx	E coli, S. cerevisae, P. pastoris and their derivatives	no	<1%	pTDH3, pCCW12, pPGK1, pHHF2, pTEF1, pTEF2, pHHF1, pHTB2, pRPL18B, pALD6, pPAB1, pRET2, pRNR1, pSAC6, pRNR2, pPOP6, pRAD27, pPSP2, pREV1, pMFA1, pMFa2, pGAL1, pCUP1, pLac	*Gluconacetobacter xylinus, G. lucidum, T. versicolor, A. bisporus, P. ostreatus,* and *P. microspor,* S. cerevisae, P. pastoris and their derivatives
Shuttle vectors, derived from pUC19	E. coli	no	<10%	pLac, bla, GPD from G *lucidum,* J23104, synthetic	*Gluconacetobacter xylinus, G. lucidum, T. versicolor, A. bisporus, P. ostreatus,* and *P. microspor*
Plasmids: F, P1, pSC101, R6K, (pRB322, pBBR1, P15a, pMB1, colE1, RSF1050, R6K and derivatives	E coli	no	<1%	P_Y, pTraM, tac, T7, SP6, BAD, lac, OmpC, FixK2 and CGG	NA
M13	E coli		100%	8 native promoters s.n.	NA

2. If using viral vectors, fill out the table below. If using multiple types of viral vectors, please add a new table per vector type; multiple variations of the same viral vector type can be in the same table. (If using retroviral or lentiviral vectors additional requirements apply. Please see MIT CAB/ESCRO policy at the following URL: http://web.mit.edu/cab/policies.html N/A

c. Use of Animals (including invertebrates) or Insects: Check box if not applicable ☒
Most research using animals also needs the approval of the MIT Committee on Animal Care (IACUC). Contact them register your animal research. The questions below are intended to deal with the use of potentially biohazardous agents in animals/insects or the creation of transgenic animals/insects.

1. Will rDNA technologies (such as producing transgenics), recombinant microbes/cells be used in animals or insects? Yes ☐ No ☐

   a. If yes, please describe objective of the research. Include recipient species, and list pathogen, rDNA technology, or recombinant microbe or human materials that will be used in animal/plant/ insect, and resulting genotype. What selection marker will be used?

2. If transgenic, including “knockout”, animals/insects will be generated, please provide information on the transgene and vector as well as the expected phenotype of the recombinant animal/insect.

3. Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory or animal facility? Yes ☐ No ☐ If yes, explain:

d. Large-Scale Research: Check box if not applicable ☒

Do experiments involve growth of 10 liters or more of culture at a time? If YES, identify culture room and type of equipment used for large-scale culture growth and handling.

e. Use of Plants: Check box if not applicable ☒

Describe experiments that involve recombinant work and plants, whether the plant itself is made recombinant or recombinant cell or microorganisms are used with plants.

Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory? Yes ☐ No ☐ If yes, explain:

Section 6. Biological Agent Use: Check box if not applicable ☐

(Please complete this section if you work with viable microorganisms or viruses.)

a. Agent identification. List biological agent(s)/microorganism(s) genus/species/strain and genotypic markers, host range; any antibiotic resistance; recommended Biosafety Level (CDC); the source of the agent (e.g. new isolate from human tissue, blood, animal, tissue culture, another laboratory, ATCC, etc.).

Genus and species	Strain	Bio safety level (BSL) / Risk group (RG)	Source	Max volume or Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Escherichia coli	DH5alpha, S17 lambda pir, MG1655, Nissle 1949, ER2738, XL1-Blue, Top10, BL21, SE5000, MC4100, C321.DeltaA	1	CGSC, Michael Fischbach (UCSF), CGSC, T. K. Lu (TKL), F. Issacs (Yale University), New England BioLabs (NEB), Agilent Technologies	1 L	10% final concentration of bleach	No

M13 bacteriophage	M13KE, E3, E4, DSPH-BAP, ENKVE-BAP, IROX8, ZNO3, Y21M, Y21G, Y21D, Y21H	1	TKL, NEB, Angela Belcher (MIT), Chris Voigt (MIT)	1 L	10% final concentration of bleach	No
Gluconacetobacter xylinus aka hansenii	ATCC53582	1	ATCC, TKL	1 L	10% final concentration of bleach	No
Ganoderma lucidum	G.260125-1	1	Chao Sun's laboratory	1 L	10% final concentration of bleach	No
Trametes versicolor	ATCC:34578, 34671, 20869, 60985, 62976; 200801, 38072, 32085, 38069, 20547,44677, 96187, 200098, 42462, 44308, 34584, 12679, 11235, 201342, 66173, and other RG1 strains from ATCC	1	ATCC	1 L	10% final concentration of bleach	No
Agaricus bisporus	MYA2386, 200037, 66256, 56372, 62471, 200083, 56378, 62468, 34847, 56439, 56439 and other RG1 strains from ATCC	1	ATCC	1 L	10% final concentration of bleach	No
Pleurotus ostreatus	90520, 34674, 38537,	1	ATCC	1 L	10% final concentration of bleach	No

	52955, 32237, 38538, 200129, 58052, 201216, 34677 and other RG1 strains from ATCC					
Pestalotiopsis microspora	ATCC 26275 ATCCMYA-4556	1	ATCC	1 L	10% final concentration of bleach	No
Brettanomyces bruxellensis	Natural isolate from kombucha	1	ATCC, Benjamin Wolfe (Tufts)	1 L	10% final concentration of bleach	No
Zygosaccharomyces bisporus	Natural isolate from kombucha	1	ATCC, Benjamin Wolfe (Tufts)	1 L	10% final concentration of bleach	No
Saccharomyces cerevisiae	W303, BY4742, YPH500	1	ATCC, TKL	1 L	10% final concentration of bleach	No
Pichia Pastoris	GS-115, Glycoswitch, ATCC 76273, X-33	1	ATCC, TKL	1 L	1% final concentration Wescodyne	No

For agents that are pathogens, please complete the following table: N/A

Are any of the biological agents used or stored in your laboratory select agents? NO Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). If yes, which ones? **N/A**

b. Experimental Procedures:

1. Describe experiment and procedures involving use of biological agent?

E. coli will be grown and transfected using standard methods, namely transfection into chemically competent cells (2002. *Escherichia coli*, Plasmids, and Bacteriophages. Current Protocols in Molecular Biology. 59:1.0:1.0.1–1.0.3.)

M13 bacteriophage will be grown as described in Greenstein, D. and Besmond, C. 2001. Preparing and Using M13-Derived Vectors. Current Protocols in Molecular Biology. 00:IV:1.15:1.15.1–1.15.8.

Gluconacetobacter xylinus aka hansenii will be grown in HS medium as described in Schramm, Gromet and Hestrim, 1957, Synthesis of cellulose by *Acetobacter xylinum*. 3. Substrates and inhibitors, Biochem. J. 67:669-679. Transformation will be by electroporation, starting with conditions used for E. coli, and optimizing from there.

S. cerevisiae, *Brettanomyces bruxellensis,* and ***Zygosaccharomyces bisporus*** cultures will be kept at -80ºC for long term storage in 1.5mL vials containing 17% glycerol. S. boulardii will be plated from frozen glycerol stock on 1xYPD-agar plates or YNB-agar plates missing the corresponding auxotrophic marker (His, Ura, Trp, Leu). Colonies from plate will be inoculated in liquid media, either 1xYPD or YNB missing the corresponding auxotrophic marker. S. cerevisiae will be transformed using standard methods, namely by electroporation or the chemical LiAc method (Becker, D. M. and Lundblad, V. 2001. Introduction of DNA into Yeast Cells. Current Protocols in Molecular Biology. 27:III:13.7:13.7.1–13.7.10).

Pichia pastoris - Electroporation and growth: Competent cells were prepared by first growing a single colony of P. pastoris in 5 mL yeast extract peptone dextrose (YPD) at 30°C for 48 hours. 100 µL of the resulting culture was inoculated in 50 mL of YPD and grown at 30°C for another 24 hours. The cells were centrifuged at 1,500 g for 5 min at 4°C and resuspended in 50 mL of ice-cold sterile water, then centrifuged at 1,500 g for 5 min at 4°C and resuspended with 20 mL of ice-cold sterile water, then centrifuged at 1,500 g for 5 min at 4°C and resuspended in 10 mL of ice-cold 1 M sorbitol, and then centrifuged at 1,500 g for 5 min at 4°C and resuspended in 0.5 mL of ice-cold 1 M sorbitol. 5 µg of plasmids of interest and 5 µg of Bxb1 recombinase expression vector were mixed, then added to 80 µL of competent cells and incubated for 5 min in an ice-cold 0.2 cm electroporation cuvette. Pulse parameters were 1,500 V, 200 Ω, and 25 µF. Immediately after pulsing, 1 ml of ice-cold 1 M sorbitol was added to the cuvette, and the cuvette content was transferred to a sterile culture tube containing 1 mL 2× YPD. The culture tubes were grown overnight at 30°C at 250 rpm. Samples were then spread on YPD agar plates with 75 µg/ml zeocin.

Ganoderma lucidum, Trametes versicolor, Agaricus bisporus, Pleurotus ostreatus, and ***Pestalotiopsis microspora*** will be grown on potato dextrose agar plates. The plates will be stored at 4ºC. Cubic hyphae will be transferred to fresh plates every two months. The transformation will be performed using restriction enzyme mediated integration (REMI) (Kim, S., Song, J. & Choi, H. T. Genetic transformation and mutant isolation in Ganoderma lucidum by restriction enzyme-mediated integration. FEMS Microbiol. Lett. 233, 201–204 (2004)) or electroporation.

2. Will experiments result in acquisition of new characteristics such as enhanced virulence, infectivity, drug or antibiotic resistance, or change in host range? Yes ☐ No ☒ If so, explain:

3. Will you introduce hazards (such as microorganisms or cells) into animals? Yes ☐ No ☒ If so:

   a) Please describe:

   b) List your MIT Committee on Animal Care (IACUC) protocol no(s), if your animal model also needs MIT Committee on Animal Care (IACUC) approval: _____

Section 7. Use of Human Source Material: Check box if not applicable ☒

a. Do you have an Exposure Control Plan (ECP) on file with the MIT EHS office? YES___ NO___

b. If no, then how has the material been treated prior to use in the lab (such as formalin fixing or heat treatment)? Please describe how material will be rendered noninfectious prior to use.

Section 8. Human Embryonic Stem Cells or Induced Pluripotent Stem Cells (hES or iPS Cells respectively): Check box if not applicable ☒

Investigators should be aware of the NIH Guidelines on Human Stem Cell Research if they plan to work with either cell type (http://stemcells.nih.gov/policy/2009guidelines.htm).

1. **Do you plan to derive human embryonic stem (hES) cells?**
   Yes ☐ No ☐ If yes, please describe the technology e.g. single cell nuclear transplantation, derivation from a donated embryo, etc. Please contact the Biosafety Office at 2-3477 as we will need further information. Please note that NIH will not fund derivation of hES cell lines.

2. **Are the human embryonic stem cells (hES) with which you plan to work on the NIH Registry of federally approved lines?** Yes ☐ No ☐ If yes, please list cell line(s) and indicate where you will get the hES cell line.

3. **If you plan to use an already existing human stem cell line that is not on the NIH Registry please provide the following information as an attachment to this Biological Research Registration:**
   a. Please list hES Cell Lines and source: another laboratory or investigator
   b. Documentation required as part of registration (from the source investigator and institution)
      i. Please submit a Letter of Assurance from the investigator supplying the cells. This letter should document that the hES cell lines were generated with Institutional Review Board

(IRB) oversight and approval. Please be sure that the source investigator includes the name of the approving IRB and the IRB OHRP assurance number.

ii. Please submit the approval letter from the Institutional ESCRO Committee.

c. MIT investigators must document that a source of non-federal funding for research involving these particular hES cell lines is in place.

d. A plan must be developed to ensure separation of supporting materials and equipment for work with all non-federally approved hES cell lines.

e. Contact the Office of Sponsored Programs for information on funding issues surrounding research involving non-federally approved hES cell lines.

4. **Funding Information: Please list all grants and contracts, including pending grants or contracts, that describe the use of any hES cell based research. Please include funding source e.g., NIH, HHMI, JDRF, etc., and start and end dates. If departmental or MIT funds are used please indicate that as well (enter "internal MIT funds" into grant/contract space).** (The box is expandable.)

Funding Agency	Grant/ Contract Number	Internal Cost Object	Date Grant Submitted	Date Grant begins	Date Grant ends

Does the information in this biological research registration form include all of the laboratory research section(s) described in the grants or contacts listed above?

☐ Yes ☐ NA (internal MIT funds to support this research only) ☐ No (Explain)

5. **Will you only receive iPS cells from a vendor or collaborator in an induced form?** Yes ☐ No ☐ If yes, please state the method of induction.

   If viral transduction is used, please state whether any residual virus is expected to be present in the induced cells.

6. **Do you plan on isolating and developing human induced pluripotent stem (iPS) cells or cell lines?** Yes ☐ No ☐ If yes, please describe the source of the somatic cells to be used and complete Section 7 of this form. In addition you will need to develop an Exposure Control Plan. Is the donor traceable? Yes ☐ No ☐

7. **What methods will be used to generate the induce pluripotent stem cells?** If you use viral vectors to generate the iPS cells please complete the detailed questions in Section 5 of this form.

8. **Do you plan to inject or transplant hES or iPS cells in any animal, animal embryo or at any stage or prenatal development where the hES or iPS cells may contribute to the animal germline?** Yes ☐ No ☐ Please outline the animal research in the space below.

Section 9. OCCUPATIONAL HEALTH ASSESSMENT, MEDICAL SURVEILLANCE & MONITORING: Check box if not applicable ☒

Some research may involve the need for a health assessment or vaccination prior to the initiation of the project. If there is a health risk associated with this research, please check the appropriate box below and contact Occupational Medicine. You can contact Occupational Medicine at 617-253-8552 to arrange

an appointment. Occupational Medicine assessment is available to all MIT employees/investigators regardless of the biosafety level of the research

- ❑ **Pre-project serum samples.** These samples of blood serum are collected prior to beginning work with some types of infectious materials to serve as a reference should an infection occur during the course of work with an agent.

- ❑ **Pre-project vaccinations other than the Hepatitis B vaccine.** A vaccination may be warranted based on the nature of the work being done and the availability of an appropriate vaccine. Check the box if you need a vaccine other than Hepatitis B. Type of Vaccine:_____

- ❑ **Medical Surveillance monitoring**. This may include a baseline assessment, periodic evaluations during the experiment time period, and a final evaluation at the end of the experiment. **Note:** This type of surveillance is not usually indicated for research below Biosafety Level 3.

Section 10. Use and/or Storage of Toxins: Check box if not applicable ☒

This section reflects requirements at the federal and Institute level regarding risk assessment, training, procurement, use, storage and disposal of biological originated toxins used in research.

a. Please describe the specific use of toxin(s) in your research. Provide enough information to understand how the toxin will be handle in terms of safety and security.

b. Please complete the following table for each Select Toxin used.

Select Toxin name	Serotype	Proposed max. qty. at all times (mg)	Research Use (concentration in dilutions)		
			In-vivo	In-vitro	Other (please write in)
Abrin					
Botulinum neurotoxins					
Conotoxin					
Diacetoxyscirpenol (DAS)					
Ricin					
Saxitoxin					
Staphylococcal enterotoxins					
T-2 toxin					
Tetrodotoxin (TTX)					

1. Please describe the specific use of toxin(s) in your research. Provide enough information to understand how the toxin will be handle in terms of safety and security.

2. For current approved holders of select toxins, are you storing select toxins in your laboratory space, but not actively using them? Yes ☐ No ☐ If yes, you are still required to keep these materials accounted for and registered in this BRR.

3. Please complete a Standard Operation Procedure (SOP) template can be found here (https://ehs.mit.edu/site/content/select-agent-toxins). Please complete, print and share this SOP with the users to provide steps to the safe handling of these products.

c. Please complete the following table for each biological toxin or component (e.g. toxoid, subunit) of a biological toxin. This table covers toxins that are not regulated but that may represent a risk to personnel and/or the laboratory environment.

Toxins name	Subunit/ Toxoid name	Source organism	vendor	LD_{50} (mg/kg)	Toxicity mechanism of action and Web link to product information or SDS
Diphtheria					
Cholera					
Shiga					
Aflatoxin					
Chlorotoxin					

Note: No SOP is required for biological toxins that are not select toxins. A written procedure is recommended for the lab to maintain locally reflecting consistent levels of safety practices and methods to properly handle, store and dispose of these products.

d. What protocol will be used to inactivate the toxins? List the toxins* under the select method(s):

☐	20% bleach (1.0 % NaOCl) 30 minutes:	☐	50% bleach (2.5% NaOCl) 30 minutes:
☐	50% bleach (2.5% NaOCl) + 0.25 N NaOH 30 minutes:	☐	10% bleach (0.5 % NaOCl) 30 minutes:
☐	Autoclave:	☐	Other (please describe):

(*) please see https://ehs.mit.edu/site/content/disposal for specific information about inactivation methods.

Section 11. Dual-Use Assessment (required): ☐ Yes ☒ No. If yes, please indicate which question number and answer below.

"Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. The United States Government's oversight of DURC is aimed at preserving the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research." (NIH Office of Science Policy)

a. Do you work with or have any of the following 15 agents or toxins in storage? Yes ☐ No ☐

	Avian influenza virus (highly pathogenic)		Marburg virus
	Bacillus anthracis		Reconstructed 1918 Influenza virus
	Botulinum neurotoxin		Rinderpest virus
	Burkholderia mallei		Toxin-producing strains of *Clostridium botulinum*
	Burkholderia pseudomallei		
	Ebola virus		Variola major virus
	Foot-and-mouth disease virus		Variola minor virus
	Francisella tularensis		*Yersinia pestis*

b. If you answered yes for any of the boxes in (a) above, are your experiment(s) expected to:

1. Enhance the harmful consequences of the agent or toxin, such as result in acquisition of new characteristics such enhanced virulence, infectivity, stability, transmissibility, or the ability to be disseminated? Yes ☐ No ☐ If so, explain:
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification? Yes ☐ No ☐ If so, explain:

3. Confer to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies? Yes ☐ No ☐ If so, explain:
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin? Yes ☐ No ☐ If so, explain.
5. Alter the host range or tropism of the agent or toxin? Yes ☐ No ☐ If so, explain:
6. Enhance the susceptibility of a host population to the agent or toxin? Yes ☐ No ☐ If so, explain:
7. Generate or reconstitute an eradicated or extinct agent or toxin listed in (a), above? Yes ☐ No ☐ If so, explain.

c. If you answered yes for any of the boxes in (a) above, are the strains used attenuated? Yes ☐ No ☐ **If so, explain:**

d. If you answered no to all of the boxes in (a) above, are your experiment(s) expected to result in the development of materials or technologies with "dual use" potential listed in (b)? Yes ☐ No ☐ **If so explain:**

e. If your research does no use any of the materials listed in (a) above and you answered "No" to (d) above, then check the "No" box at the top of Section 11.

Section 12. Safe Practices and Procedures (required): Please complete this section for all viable biological research agents or materials including human-derived materials.

1. Please identify and discuss the health and safety risks associated with the proposed research use of this biological agent or recombinant materials. (Please refer to Sections above if relevant):

   Neither Risk Group 1 (RG1) bacterial strains, RG1 yeast strains, mushrooms nor bacteriophage used in the lab are known to cause disease in health adult humans and are all safely used following BL1 practices and procedures.

   M13 bacteriophage is a pathogen for bacteria.

2. What procedures create the greatest risk of exposure or infection e.g. aerosolization of materials, and how will this risk be minimized during the course of the research:

   No research involves processes highly likely to aerosolize materials, eg. Waring Blender. Work with potentially infections materials will be performed in a biological safety cabinet or in a sealed anaerobic chamber;

3. Outline protective equipment required to minimize exposure of laboratory personnel during all procedures requiring handling or manipulation of biological agent e.g. use of gloves, lab coats, safety glasses, etc.

☒	Lab Coat	☒	Gloves
☒	Safety glasses - researchers are instructed on which activities require use of safety glasses.	☐	Safety Goggles
☐	Face shield (please specify procedure):	☒	Other (Please describe PPE and procedure): closed toed shoes and long trousers/skirts

4. Outline decontamination procedures and disinfectant(s) to be used for work surfaces, instruments, equipment, liquid containing biological materials and glassware:

Liquid waste decontamination	
☒	10% final concentration of household bleach (0.5% NaOCl), 20 minute contact time
☒	Wescodyne (1%), 20 minute contact time
☐	Autoclave (Please describe rationale):
☐	Other (Please describe rationale):
Work surfaces and equipment decontamination	
☒	10% final concentration of household bleach (0.5% NaOCl)
☐	70% ethanol
☒	EPA approved product such as Skalr, Lysol spray, PREempt, etc. (Please list product(s)):
☐	Other (Please describe):

5. Outline disposal/decontamination procedures for contaminated sharps, contaminated solid waste, tissues, pipette tips, etc.

☒	Solid waste	Placed in EHS provided biowaste boxes for disposal.
☐	Solid Waste	Autoclave (please describe rationale):
☐	Animal or human tissue	Placed in EHS provided biowaste boxes dedicated for incineration
☒	Sharps	Placed in puncture resistant biosharps container and dispose of full containers in EHS provided biowaste box.
☐	Other (Please specify):	Describe process:

6. Will mixed waste be generated (radioactive/biological or chemical/biological)? YES ☒ NO ☐
If yes, please indicate how you will inactivate the biological component of the mixed waste in the box below. (For information on waste management, please see: http://ehs.mit.edu/site/waste. If you have questions, contact EHS at 2-3477).

   We will use Wescodyne (2-3% final concentration) to inactivate P. pastoris.

   For experiments with nanoparticles and/or quantum dots, the biological will be inactivated to final concentration of 10% bleach or Wescodyne (2-3% final concentration) for 20 minutes and then collected as hazardous waste.

Section 13. Certification and Signatures

The information contained in this application is accurate and complete. I am familiar with and agree to abide by all guidelines and regulations pertaining to this research. These guidelines and regulations include the current NIH Guidelines for Research Involving Recombinant DNA Molecules; CDC and NIH guidance documents such as “Biosafety in Microbiological and Biomedical Laboratories”; the DHHS and USDA Select Agents and Toxin regulations; OSHA Bloodborne Pathogen Standard; the provisions of the City of Cambridge Ordinance on Recombinant DNA Research; the Massachusetts State Sanitary Code Chapter VIII, 105 CMR 480, “Minimum Requirements for the Management of Medical or Biological Waste; Massachusetts law, M.G.L. c.111L, “Biotechnology” for human embryonic stem cell research; as well as any MIT Policies and Procedures and other local, state and federal regulations that may be applicable.

Specifically I agree to abide by the following requirements:

a. I will not initiate any biological research subject to the regulations and guidelines mentioned above until that research has been registered, reviewed and approved by the Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight (CAB/ESCRO). The purview of the MIT CAB/ESCRO includes biological research involving recombinant DNA; biological agents and pathogens; human cells, tissues, materials and embryonic stem cells; select agents and toxins, and the use of any of the above in animal research.
b. I will assure that personnel have received appropriate information about the biological hazards of the research outlined in this registration by making available copies of approved protocols, Biosafety Manuals, and Biological Research Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.
c. I am familiar with and will ensure use of appropriate biosafety level laboratory practices and procedures in the conduct of this research.
d. I certify that laboratory personnel have appropriate technical expertise.
e. I will ensure that laboratory personnel know the procedures for dealing with incidents and spills of biological materials, and know the appropriate waste management procedures.
f. I will comply with all shipping requirements for biohazardous materials.
g. I will ensure that all laboratory personnel working with biological materials are listed on this registration.
h. I will assure that all laboratory personnel have completed all necessary training and that their training records are up to date.
i. I certify that all laboratory spaces associated with the research described in this registration are listed.
j. If this research involves recombinant or synthetic nucleic acid technologies, I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the “NIH Guidelines for Research Involving Recombinant DNA Molecules” (a copy of this section is available from the MIT Biosafety Program)
k. I will assure adequate supervision of personnel, and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.
l. I will inform the MIT Biosafety Program of any serious spills, potential exposures or breaches of the guidelines and regulations listed above.

_____ _____

Principal Investigator Date

_____ _____

MIT Biosafety Officer Date