PROJECT APPLICATION FOR THE HUANG-HOBBS BIOMAKER SPACE

Thank you for your interest in the Huang-Hobbs BioMaker Space. In using the shared space, all biomakers must do their part to ensure that BioMaker Space activities have zero impact on other users and the facilities. As such, you must take very seriously your obligation to use BioMaker Space facilities responsibly and respectfully at all times. All users are required to take appropriate training before using the space and equipment within the space. All projects in the BioMaker Space are subject to ongoing approval by staff and MIT EHS (Environment, Health, and Safety). Access may be terminated at any time and without notice.

Please answer the questions in this application so we may evaluate your project's suitability for the BioMaker Space. This application is under development. Let us know how we can improve it!

Project Review Process:

There are two stages to the application process for new projects. The first stage is the "Mission Application" in which applicants provide basic information about themselves, teammates, and the general nature of the project to be performed in the space. This application is reviewed by a panel of faculty, staff, and students to assess the fit of the project with the space capabilities and the intended purpose. If capacity is limited, this application serves as the basis for prioritization of projects invited to submit technical applications. The Stage 1 Application is completed using an online form with a series of selection and free response questions. Applicants must submit enough information to communicate the nature of the project and the team to the review panel, but the most detailed technical and proprietary information may be omitted at this stage.

All projects are subject to review and reapproval on an ongoing basis. Projects which wish to maintain access to the space for upcoming terms must submit their renewal request through the Stage 1 Application and provide updates on project progress and resource availability.

New project applications selected during the Stage 1 Application will be invited to submit a full "Technical Application". The Stage 2 Application will be reviewed by staff of the BioMaker Space, a designated faculty expert for the project subject matter, the departmental EHS Coordinator, and staff from the central EHS Office. The Stage 2 Application must include all biological materials to be used in the project (e.g. strains, cell lines, tissues and primary biological materials, proteins, nucleic acids, etc.) as well as all hazardous chemicals and equipment. Applicants must submit "pseudo protocols" with sufficient detail for review. Continuing projects do not need to resubmit the Stage 2 Application unless there are changes to the project scope; applicants are required to inform staff of the BioMaker Space and EHS of any material changes to materials or procedures of the project.

The BioMaker Space operates with three terms over the academic year. The fall term covers September – December; IAP/Spring term covers January – May. The summer term covers June – August. Each term has a standard application deadline for new projects to ensure prompt registration of projects and access to the space. Applications after the deadline MAY be considered at later deadlines and/or on a rolling basis at the discretion of the staff based on capacity and availability of reviewers.

A Note on Confidentiality:

The BioMaker Space will strive to keep information in the Stage 1 Application to as limited of an audience as possible, but it cannot guarantee confidentiality; do not disclose proprietary information on the Stage 1 Application. BioMaker Space staff and the review panel cannot sign non-disclosure agreements.

Details contained within the Stage 2 Application will be kept within a very limited audience to protect intellectual property, and applicants may ask about who will see this information. The goal of the staff is to enable projects to be performed safely in the BioMaker Space and with the greatest chances for positive outcomes, and all staff are committed to safeguarding intellectual property. Applicant information will not be distributed beyond necessary staff and, because all staff are MIT employees, our interpretation is that an application does not constitute a public disclosure. If you are uncertain or uncomfortable disclosing any information, it is recommended you seek professional legal advice prior to completing this application.

BioMaker Space staff are not authorized to enter into agreements on behalf of MIT; the policy of the BioMaker Space is that staff will **not** sign non-disclosure agreements directly with applicants *in their role and capacity as BioMaker Space staff*. While staff will protect the confidentially of information and will not distribute materials outside of the application review and registration process, applicants should not reveal materials that are deemed overly sensitive and proprietary (e.g. trade secret). Staff will work with applicants to limit details to those required for review and MIT registration purposes. Applicants are free to execute non-disclosure and confidentiality agreements on an individual basis with project mentors and advisors, but these agreements are outside of the role and capacity of the BioMaker Space project approval and supervision.

Pre-Application / Renewal Request (IMPORTED FROM ONLINE FORM)

Project title: Bacterial Leather Bioengineers

Term: Summer
Year: 2024

Section A. Participants

1) Participants:
   a. Point of Contact

Full Name	Morgan Guempel
Kerberos	mguempel
MIT ID	923922662
Current MIT Affiliation	Undergraduate
DLC Affiliation	Course 20
Alternate Email	mguempel@mit.edu
Phone Number	0

   b. Has the point of contact changed? No

   c. Other Participants

      Yitong Tseo, yitongt@mit.edu, Computational Systems Biology PhD Student

   d. Have participants been added or removed? No

Section B. Project Summary

2) Project goals in layman terms:

Komagataeibacter rhaeticus is a naturally cellulose producing strain of bacteria - the cellulose it produces has been shown to be quite mechanically robust and we are excited to find applications for it as a cruelty free, sustainable leather substitute. To that end we want to engage in a project with two goals:
* Modifying K. rhaeticus to secrete a hydrophobin protein (BslA) to hopefully increase the hydrophobicity of the "bacterial leather"

* Create a genetic circuit to produce chitin instead of cellulose in the presence of certain wavelengths of light via a light sensitive inducer. Then we can build a special programmable bioreactor for the bacteria to create patterns of different mechanical strength.

3) Please list any MIT activities related to this project:

UROP

4) Is this a continuing project or a new project application? If continuing, please describe your progress over the past term

New

5) Please list your objectives for the upcoming term

As part of the UROP, we have facilities for Komagataeibacter rhaeticus culturing and mechanical workspace for building the novel bioreactor. Our goal for the upcoming term is to clone the E. Coli in the Huang-Hobbs lab space (electroporation, golden gate

6) Please provide an update on your available funding

I will have complete funding for the project from the Bioinstrumentation lab! Just need to access the lab space.

7) How many person-hours do you anticipate using the space per week this term?

5 hr/wk

8) Will you plan to use the tissue culture room? If so, how many hours per week in the tissue culture room?

No
hr/wk

9) Would you like to discuss making this into a BioMaker Space training workshop?

Maybe

10) Would you like to discuss making this a community project?

Yes

11) Are you willing to allow the BioMakerspace to use information from this application and about this project for the propose of promoting the space in media and to potential sponsors?

Yes

12) Additional comments or questions:

Technical Application Sections

Section 1: experimental plan

1.1. Project details in scientific terms: [~1/2 page]

Aim 1: Induced surface hydrophobicity by expressing hydrophobin proteins

Cellulose is naturally hydrophilic, and readily absorbs water to become a hydrogel. However this property is not desired for use cases in fashion and building materials (Nam 2018). This leads us to the idea of genetically modifying Komagataeibacter to produce a hydrophobic coating which will protect the bacteria leather from absorbing water. Building off of the Komagataeibacter tool kit (KTK) (Goosens 2021) we have designed a plasmid containing BslA, a hydrophobin protein, in conjunction with a cellulose binding module (CBM). This plasmid additionally encodes transport machinery and the correct signaling peptide for extracellular excretion of the BslA-CBM construct. In previous work BslA-CBM constructs introduced into cellulose were found to significantly increase water deflection (Gilmour 2023). If successful, our approach will represent the first time the hydrophobic coating is endogenously produced by the Komagataeibacter itself and should present a simpler and more scalable system.

Aim 2: Optogenetically induced chitin-cellulose copolymerization

Chitin is the second most prevalent polymer in the world, only less prevalent than cellulose, and forms the naturally tough backbone of shellfish shells, fungi cell walls, and insect exoskeletons. We want to explore linking chitin production in Komagataeibacter to an optically inducible genetic circuit so that we can tune the mechanical properties of the resulting bacterial leather in the spatial dimension. An initial plasmid has already been designed linking a Komagataeibacter tailored optogenetic trigger (Walker 2024) to Komagataeibacter chitin production machinery (Yan Teh 2019). Competent E. Coli cell stock as well as all the DNA fragments have been purchased from NEB and Twist. Golden gate cloning has been greatly facilitated again by the KTK (Goosens 2021). We are also in the process of designing a lightbox bioreactor that is capable of projecting patterns onto the growing bacterial leather activating the optogenetic machinery. The final goal of the project will be to build a “living” 3D printer which prints patterns of chitin supported in cellulose scaffolding.

1.2. Research strategy & Pseudo-Protocols:

See attached spreadsheet of all the different DNA fragments for the project. Following standard golden gate cloning protocol, we intend to create plasmids in Turbo competent E. Coli. (Protocol). Specifically the golden gate framework makes use of type IIS restriction enzymes (BsaI and BpiI) to cut outside their recognition site, creating unique 4-base overhangs. And DNA ligase to fuse matching fragments together.

The KTK toolkit includes all requisite promoters, ribosome binding domain, and terminator sequences (Goosens 2021). So only DNA fragments of the active machinery parts are specially ordered from Twist.

After plasmids are constructed what's next is to transform them into the K. rhaeticus iGEM strain. There are several protocols to make the strain electrocompetent (Florea 2016, protocol in supplemental) which we intend to follow.

To confirm the plasmids were transformed correctly we intend to do a HIS-tag purification with a Nickel column (we made sure to include His-tags on the proteins of interest).

Finally all that's left is to culture the genetically modified K. rhaeticus in a bioreactor of our design. And to quantify its mechanical / hygroscopic properties again with tensile test apparatus and the droplet angle analysis.

Section 2: team members & training

2.1. Please list all team members applying to work in the BioMaker Space

Laboratory Personnel					Research Materials Used (place an ☒ in the appropriate box)			Training Completed (Enter most recent date of training)			
Name	Kerberos	MIT ID	MIT Affiliation	UROP (Y/N)	Uses material at BL1	Uses material at BL2	Uses human-derived material	General Biosafety training	Bloodborne Pathogens training	Relevant Course Work (e.g. 7.002, 7.003, 7.102, 10.28, 10.29, 20.109, 20.129, 20.309, 20.345)	Other Qualifications (e.g. UROP / prior research experience)
Morgan Guempel	mguempel	923922662	Undergraduate (Course 20)	Yes	☒	☐	☐	2022	2022	20.109, 20.309, 20.305	2023: Weng Lab at Whitehead, plasmid construction and transformation in *E. coli* and yeast 2024: Bioinstrumentation Lab designing plasmids and cloning *in E. coli* (so far)
Yitong Tseo	Yitongt	924636742	PhD Student	No	☒	☐	☐	2023 (not sure of the exact month since it seems the training lasts forever)		20.201, 7.58 7.95, CSB.100, 1.545, 6.4842	Genetics & Genomics Certificate at Stanford, Bachelors in chemistry fron

											Williams College 4 years of research in th Facebook Healthcare team
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				
				Y/N	☐	☐	☐				

Section 3: mentorship and feasibility

3.1. Who has advised you so far?

Yitong Tseo – a PhD student and mentor for the UROP project. Dr. Cathy Hogan a senior research scientist in the Bioinstrumentation lab. Additionally, Dr. Felix Radford – a postdoc in George Church's group at Harvard who is a close collaborator in this project.

3.2. List any potential mentors you have identified at MIT who you could turn to for expert advice.

PhD student Ceili Peng in the Engineered Evolution group at the Media Lab.

3.3. Would you like support in identifying and approaching mentors?

Perhaps, but currently feel well supported.

3.4. Please list potential candidates for supervising your work in person if you need to access the BioMaker Space during non-standard hours.

Yitong Tseo, Dr. Justin Buck

3.5. List peer-reviewed literature that suggests the feasibility of your project.

Goosens V. J., et al. Komagataeibacter Tool Kit (KTK): A Modular Cloning System for Multigene Constructs and Programmed Protein Secretion from Cellulose Producing Bacteria. ACS Synth. Biol. 2021, 10, 12, 3422–3434. 2021

Gilmour K. A., et al. Biofilm inspired fabrication of functional bacterial cellulose through ex-situ and in-situ approaches. Carbohydrate Polymers, Volume 304, 2023.

Walker, K.T., et al. Self-pigmenting textiles grown from cellulose-producing bacteria with engineered tyrosinase expression. Nat Biotechnol (2024). https://doi.org/10.1038/s41587-024-02194-3

Teh, M. Y., et al. An Expanded Synthetic Biology Toolkit for Gene Expression Control in Acetobacteraceae. ACS Synth. Biol. 2019, 8, 4, 708–723, 2019

Section 4: budget and funding

4.1. Include a detailed budget, with justification.

Likely this project will not need a budget, as funding is being supplied through the UROP. What would be very helpful would be access to shared feedstocks of common laboratory reagents (Tris buffer, Arabinose, PIPES, etc.) and access to laboratory equipment. If there's anything missing I can ask Yitong to purchase it through the lab funds.

4.2. Have you secured or are you seeking funding for this project? If yes, list source(s) and amount.
We have secured funding for this project through the Bioinstrumentation laboratory: ~$5000 worth.

4.3. Are any of your team members financially compensated for their work in the BioMakerspace? If yes, please specify by what entity.

Morgan Guempel is paid through the summer UROP program

4.4. Are you applying to or have you already received any BioMaker Space funding programs? If yes, please specify which.

Nope

Section 5: intellectual property

Use of the Huang-Hobbs BioMaker Space does not constitute "significant use" of institute resources. As such utilization of the BioMaker Space does not add further restrictions or place any additional claims onto the intellectual property of project teams. However, use of the Huang-Hobbs BioMaker Space does not absolve limitations, restrictions, or claims to intellectual property based on other agreements and prior art. You are responsible for obtaining any and all permission that may be necessary to execute your project in the space. **Failure to do so may put your intellectual property at risk.** The questions below are intended to help you and BioMaker Space staff identify possible encumbrances on the intellectual property of your project. The goal is to help users of the space to achieve their objectives without impinging their intellectual property assets. BioMaker Space staff and MIT TLO are willing to help you with questions, but cannot offer legal advice regarding your IP. If you are interested in commercializing your technology, it is recommended you review your IP situation with a professional IP lawyer.

5.1. Is any part of this work an extension of another MIT research project?

Yes this is part of a UROP project that will continue into the Fall.

5.2. Do you have all of the rights needed to work on this project (e.g., are you using an MIT-owned biological material) and/or would you like to speak to the Technology Licensing Office (TLO) about obtaining rights?

Yes we have all of the rights to work on this project. All of the DNA and cell strains are in the public domain.

5.3. Do you intend to maintain ownership of the intellectual property from this project?

Yes

5.4. Does any entity outside of the project team or MIT have an interest in the intellectual property of this project?

Nope

5.5. Is any portion of your project funded by a government agency?

Nope

5.6. Do you have any agreements (e.g. IP assignment clauses of employment contracts, non-compete agreement) from previous or current employment?

IP agreements with the Bioinstrumentation lab, and whatever agreements MIT has with the Bioinstrumentation lab.

Section 6: safety considerations and registration information

Biosafety

The BioMaker Space is a BL2 facility. Only work suitable for BL1 or BL2 containment can be performed in the BioMaker Space; BL2+ containment level work is not permitted. Work with live vertebrates is not permitted in the space, but tissues, cells, and primary materials from outside sources are allowed as are established cell lines. Please answer the following questions about the biological materials you plan to use and complete the following tables as relevant

6.1. Will you be using any microorganisms?

If yes, complete the table below:

Microorganisms (bacteria, viruses, fungi, parasites, protozoa, etc.)									
Genus and species	**Strain**	**Bio safety level (BSL)**	**Risk group (RG)**	**Source (vendor name or collaborator name/institution?)**	**Max volume used**	**Pathogenicity**	**Route of exposure (if applicable)**	**Known clinically relevant drug resistance**	**Special equipment /PPE used (above standard PPE)**
Example: Pseudomonas aeruginosa	**-**	**BL2**	**RG2**	**ATCC**	**500 ml**	Human pathogen	**Direct contact, inhalation**	**None anticipated**	**Biosafety cabinet**
Escherichia coli	Turbo competent	BL1	RG1	NEB	<100 mL	No	None anticipated	None anticipated	None
Komagataeibacter rhaeticus	iGEM	BL1	RG1	ATCC	500 mL	No	None anticipated	None anticipated	None

Komagataeibacter xylinus	CMCC 2618	BL1	RG1	ATCC	500 mL	No	None anticipated	None anticipated	None
		Choose an item	Choose an item			Choose an item			Chose an item
		Choose an item	Choose an item			Choose an item			Chose an item
		Choose an item	Choose an item			Choose an item			Chose an item
		Choose an item	Choose an item			Choose an item			Chose an item

6.2. Will you be using biological materials from animal sources? If yes, complete the table below:

Confirmed we will NOT be.

6.3. Will you be using biological materials from plants? If yes, complete the table below:

Confirmed we will NOT be.

6.4. Will you be using biological materials from Human Sources? If yes, complete what materials and the relevant following tables:

Confirmed we will NOT be.

6.5. Are you using any recombinant DNA? Are you planning to use any viral vectors? If yes, complete the relevant table(s) below:

Confirming yes we will be using rDNA. Confirming we will not be using viral vectors

Nature of insert or gene target	**Vector nucleic acid cloned into**	**Organism the sequence is from and Risk Group (if known)**	**Recipient bacterial strain(s) and/or host cell(s)**	**Is there an oncogenic potential? (e.g. oncogene, tumor suppressor knock-down, etc.)**	**Is the insert a toxin or from a pathogen?**	**Is a protein expressed?**	**What percentage of total eukaryotic viral genome is in the insert?**
Example: Fluorescent proteins	pCDNA3.1	*A. victoria*, *Discosoma sp.*	E. coli k12, HEK	No	No	yes	% < 1/2
CsgG	pSEVA331 and pSEVA431	E coli	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
BslA-CBM	pSEVA331 and	B subtilis	NEB Turbo E coli, K	No	No	Yes	% < 1/2

	pSEVA431		rhaeticus, K xylinus				
araC	pSEVA331 and pSEVA431	E coli	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
p_BAD	pSEVA331 and pSEVA431	E coli	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	No	% < 1/2
T7RNAP-nMag	pSEVA331 and pSEVA431	*Neurospora crassa*	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
pMag-T7RNAP	pSEVA331 and pSEVA431	*Neurospora crassa*	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
AGM1	pSEVA331 and pSEVA431	Candida albicans SC5314	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
UAP1	pSEVA331 and pSEVA431	Candida albicans SC5314	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
NAG5	pSEVA331 and pSEVA431	Candida albicans SC5314	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
Hfq	pSEVA331 and pSEVA431	*E coli*	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	Yes	% < 1/2
UGPase targeting sRNA	pSEVA331 and pSEVA431	*K. rhaeticus*	NEB Turbo E coli, K rhaeticus, K xylinus	No	No	No	% < 1/2

6.6 Will you be working with any toxins produced by living organisms? If yes, please list the material, amount, and describe plans for managing storage and disposal.

None

Chemical safety

6.7. Chemicals to be used:

Please list all chemicals:

Chemical Name	CAS Number	Hazard Classifications	Storage Conditions	Source
PIPES				

Radiation (ionizing and non-ionizing)

6.8. Are you planning to use any lasers? If yes, please explain type (e.g. power, wavelength,):

No

6.9. Are you planning to use any radiation or radioisotopes? If yes, please list:

No

Thermal (heat or cryogen)

6.10. List here heat sources (hot plates, autoclaves, furnace/high-temperature ovens):
Hot plates and pocket PCR for heat shocks.

6.11. List any cryogen use (liquid nitrogen, dry ice):
Potentially dry ice to freeze cells.

Specialized safety

6.12. Electrical safety. List here any special electricals needs and hazards (e.g. high voltage, etc.)
Electroporation includes high voltage.

6.13. Mechanical. List here any specialized mechanical equipment and hazards:
Potentially custom engineered bioreactors.

Risk assessment and management

The Huang-Hobbs BioMaker Space is a BL2 facility. All users are required to wear laboratory coats, and safety glasses at all times. Disposable gloves are required for handling chemicals and biological materials, and goggles are required for hazardous chemicals where an elevated splash risk is present. Projects may have additional safety considerations and waste management practices. Please complete this section to identify project specific risks and explain the approach for managing.

6.14. Describe potential risks that the project poses to yourself or others in the lab. Explain what steps are taken (engineering controls, training, enhanced PPE, etc.) to assess or mitigate these risks.

This project is pretty safe, it only makes use of BSL1 organisms and engineered proteins and glycans (chitin) are natural and non-toxic. I will wear common laboratory PPE (lab coat, gloves, face mask when prudent) to reduce the risk of exposure. And all culturing work will either be done in a biosafety cabinet or on a clean and protected bench top. All waste will be properly disposed of in a biological waste container.

6.15. Please explain plans to deactivate biological materials and prevent exposure.

	Liquid biological waste decontamination	
☒	10% final concentration of household bleach (0.5% NaOCl); 20 min. contact time	
☒	Wescodyne (1%), 20 minute contact time	
☐	Other (Please describe rationale):	
	Work surfaces and equipment decontamination	
☒	10% final concentration of household bleach (0.5% NaOCl)	
☒	70% ethanol (**Note**: ethanol is not an appropriate disinfectant for work involving human materials)	
☐	EPA approved product such. Please list product(s) using the dropdowns to the right:	PREempt Chose an item.
☐	Other (Please describe):	
	Solid biowaste decontamination	
☒	Solid, non-sharp biowaste placed in EHS provided biowaste boxes for disposal	
☒	Sharp exposed to biological material placed in puncture resistant biosharps container and dispose of full containers in EHS provided biowaste box	
☐	Other (Please describe process):	

6.16. Please explain plans to collect and dispose of any chemicals requiring hazardous waste disposal.

We don't expect any protocols to generate chemical waste. But if any is generated we will dispose of it properly in the hazardous waste disposal.

6.17. Will you be generating any mixed waste (i.e. waste with more than one category of waste [biological, chemical, radiation])? If yes, please describe the type of stream and disposal method.

Nope.

	Waste stream
☒	Biological DNA and protein prep kits (such as Qiagen) waste is collected as hazardous chemical waste.

	Safety note: Kit waste should never be bleached and can be sent out without further treatment.
☒	Mixed chemical/biological waste will be inactivated with a disinfectant compatible with the chemical and then sent out as hazardous chemical waste. Please describe **chemicals** and note the **compatible disinfectant** you plan to use:
☒	Mixed radioactive/biological waste will be disinfected and then handled as radioactive waste.
☐	Other (Please describe waste and inactivation methods):

Section 7: Certification & Signature

WE CERTIFY THAT ALL OF THE INFORMATION WE HAVE PROVIDED WITHIN THE APPLICATION IS ACCURATE AND COMPLETE TO THE BEST OF OUR KNOWLEDGE. WE WILL NOTIFY THE BIOMAKER SPACE AND EHS STAFF SHOULD THERE BE ANY SIGNIFICANT CHANGES TO THE BIOLOGICAL MATERIALS, CHEMICALS, EQUIPMENT, OR PROTOCOLS.

APPLICANTS NAMES	**APPLICANTS SIGNATURES**	**DATE**
MORGAN GUEMPEL		
YITONG TSEO		

Section 8: Approvals

Biosafety Approval

Biosafety Officer	Date

EHS Coordinator Approval

Matthew Machtinger	Date

BioMaker Space EHS Rep

Justin Buck or Maxine Jonas	Date

BioMaker Space PI approval

Douglas Lauffenburger	Date