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: https://cabescro.mit.edu/home
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. To modify the checkboxes, double-click on them and select “Not checked” or “Checked”. 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	x	Other		Human embryonic stem cells		Induced pluripotent stem cells
	Plants	x	Pathogens	x	Human Materials	x	Viral Vectors	x	Nanotechnology		Toxin Use

Required information: Every Principal Investigator must complete Sections 1, 2, 3, 11, 12 and 13.
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).**

Section	Description	Status
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: **821**	Title of Registration: **Synthetic Biology for Clinical Applications - Bacteria**	
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: 617-324-8129	Lab Administrative Contact phone: 617 258 0383	

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
NE47-216	BL2 (BL2+ in preparation)	1BSC + 1 to be added			X		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
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
Inda, Maria Eugenia	inda	N	X	N/A		08/08/17	
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
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.

See below for each specific project description

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:

Project 1 – Engineered Bacteriophage Therapeutics for Antibiotic-Resistant Infections

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

Antimicrobial drug development is increasingly lagging behind the evolution of antibiotic resistance, and as a result, there is a pressing need for new antibacterial therapies that can be readily designed and implemented. To tackle this problem, we are engineering bacteriophage to fill this niche. Bacteriophage therapy for bacterial infections is a concept with an extensive but controversial history. Although there has been a recent resurgence of interest in this option, bacteriophage therapy remains an underutilized option in Western medicine due to biological challenges such as limited host range, bacterial resistance to phages, side effects of bacterial lysis, and challenges to clinical use, including regulation, manufacturing, and delivery. Recent advances in biotechnology, bacterial diagnostics, macromolecule delivery, and synthetic biology may help to overcome these technical hurdles. These research efforts must be coupled with practical and rigorous approaches at academic, commercial, and regulatory levels in order to successfully advance bacteriophage therapy into clinical settings.

We are addressing the biological problems using the tools of synthetic biology, while keeping in mind the practical issues limiting the acceptance of this therapy. We are using known phages and engineering them with new properties, such as increased biofilm degradation capabilities, different host ranges, increased or decreased reproduction rates, etc. We are isolating new phages and evaluating how they might be used in bacterial infection mitigation. In addition, we use phages as host specific DNA injection nanomachines to deliver plasmid borne DNA constructs to selected pathogens and/or members of complex microbiota. These constructs can order to alter the survival rate of the host cells, their biochemistry and metabolism, or get them to produce heterologous proteins such as recombinases.

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.

1.2 Bacteriophage engineering approaches to the development of next generation phage-based therapeutics against antibiotic resistant bacteria

We are using known phages and isolating new phages to study how they might be used in bacterial infection mitigation. We pursue two main routes. 1) We develop methods to engineer phages and confer to them non natural properties such as increased biofilm degradatipon capabilities, different host ranges, increased or decreased reproduction rates, etc… 2) we use phages as host specific DNA injection nanomachines to deliver plasmid borne DNA construct to desirable pathoegns and/or members of complex microbiota in order to alter their survivability, their biochemical properpties or get them to produce heterologous proteins such as recombinases.

For both projects we rely on a wide range of both model phages and newly isolated phages originally isolated on a wide range of both non pathogenic and pathogenic bacteria. Some of those phages may be genetically engineer to change their host targetting machinery and/or possess genes that alter bacterial viability. None are engineered to encode virulence factors and any newly isolated found to have such genes would either be eliminated from our research or engineered to remove these deleterious genes. The synthetic phages may be assembled either in non pathogenic laboratory *E. coli* with traditional phage genetics techniques or using a method we devised whereby the whole phage genome is constructed as a replicating chromosome inside of the yeast *Saccharomyces cerevisiae*.

1) We will screen recombinant phage against a wide array of bacteria, including antibiotic resistant strains, including *Salmonella* (all serovars except Typhi and Paratyphi), *Klebsiella* (*pneumoniae*, *oxytoca*), *Shigella* (*flexneri*, *dysenteriae*, *boydii*), pathogenic (EHEC, STEC, EPEC, ECOR collection) and non-pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia* (*pseudotuberculosis* and *enterocolitica*), *Vibrio cholerae*, *Enterobacter cloacae, enterococcus,* and *Clostridium difficile*, in search of phages that have acquired new host specificities and/or have better/worse killing characteristics. We focus on these targets because they are responsible for a growing amount of antibiotic resistant, hard to treat, pathology and/or are easy to manipulate models that allow establishement of design principles and proof of concepts studies.

2) We will leverage advances in phage host range engineering obtained from the previous project to create a new approach to phage genome engineering. Most systems that employ phages for the delivery of DNA (transduction) use mutants that have an enhanced rate of transduction over wild-type due to either poor encapsidation of their own DNA or poor replicability of their own DNA. They therefore generate mixed populations containing phage capsids that contain the desired cargo plasmid and others that contain the virulent phage genome. Cells that receive the desired phage genome can therefore be killed by the virulent phage which leads to poor apparent DNA delivery efficiencies. We aim at creating modular syntheting Gene Transfer Agents (sGTA) that cannot produce viable phages (and ideally cannot even package their own DNA) and therefore exclusively function as gene delivery systems. Phages of the lambdoid family are naturally modular with the expression of their regulatory functions and that of their morphogenetic (head and tail) functions essentially disconnected. We will therefore create hybrids between various lambdoid phages that have a common engineered regulatory system that allows us to control when the sGTA is producing phage-like particles and various morphogenetic modules targetting bacterial pathogens of choice. We will ensure that those functions are encoded on separated discontinuous DNA pieces so that no phage-like particle may ever acquire the entire functional system and finaly create a matching array of cargo plasmid in which desired DNA circuits may be cloned for delivery and expression into the targetted pathogens. Initially we will use our sGTAs to package and inject a sequence guided nuclease plasmid designed to target essential virulence factors and/or resistance markers of *Salmonella*, *Shigella, Enterobacter, Enterococcus* or *Klebsiella*. Subsequently, we will expand the system to target natural commensals of the gut microflora in order to alter their gene expression patterns and/or provide them with capacity to produce anti-inflammatory or other beneficial activities endogenously. We will also test the capacity of the system to selectively eliminate particular genotypes from complex natural microbiomes (*Salmonella*, *Yersinia*, *Pseudomonas*, *Klebsiella, E. coli, Enterobacter*) in various animal models animal models (Mouse colonization, mouse skin infection,

wax worm injection (*Galleria mellonella*)) that have either been developped by members of the Lu laboratory or collaborators (Joan Mecsas, Tufts university). In the process, we will need to construct mutants of said pathogens that are devoid or over-express type three secretion systems as controls of the specificity and activity of our sequence guided strategy. Another application of phage mediated DNA delivery is being developed as a partnership with Piro Suiti at Novartis. The phagemid platform will be used for delivery of recombinases in *E.coli*. The work involves building phagemids for the recombinases from phages BxbI, A118, TP901, ΦC31 , and 13 other recombinases obtained from Chris Voigt's group (http://www.nature.com/nmeth/journal/v11/n12/full/nmeth.3147.html). In parallel experiments, a GFP reporter construct flanked by the recombinase recognition sites for each of these recombinases will be made and expressed in EMG2 strain of *E.coli* or BL21 containing the F+ plasmid. This is similar to experiments described in Section 7 in the paper cited above. The immediate goal is to develop a panel of M13 phages that can deliver each of these recombinases and EMG2 strains carrying their respective reporters. The future applications and pathways to be targeted and model organisms will be decided at a later date.

The bacteria and phages used in these projects have been obtained from various sources including ATCC, the Salmonella Genetic Stock Center, the Yale genetic stock center, the STEC center, isolation from natural samples and collaborators. We may need to acquire additional isolates from either natural sources or any of the above-mentioned sources or other similar stock centers. We have a particular interest in carbapenem-resistant enterobacteriaceae (CRE) and have an established collaboration with the laboratory of Julie Segre providing us with relevant antibiotic-resistant bacteria. We are also receiving samples from Rita Rahmeh and are planning on receiving some from Dr. Louis-Charles Fortier (Université de Sherbrooke) and Dr. Jeroen Corver (Leiden University Medical Center). Additionally, we will isolate novel phages to target bacteria that our current isolates are unable to target, including strains of the above-mentioned organisms as well as in a collaboration with the Fox lab to target *Enterococcus* isolates.

1.2 **Development of Therapeutic Bacteriophage for Decolonization**:

A major concern in infectious disease is the establishment of antibiotic resistance and pathogen reservoirs in healthy individuals that could potentially later cause life threatening opportunistic infections. We wish to explore the use of bacteriophage as a means to specifically eliminate antibiotic resistant or pathogenic strains from a microbiome without affecting commensal populations. We intend first demonstrate this concept using non-pathogenic *E. coli* strains (K-12, BL21, C-1) and *Klebsiella spp.* (sp. 390 and *oxytoca*). Later, we extend this concept to clinical isolates carbapenem-resistant Enterobacteriaceae (CREs), including *E. coli* and *K. pneumoniae* strains. As many of these human isolates do not naturally colonize the mouse gut, we will perturb the microbiota using antibiotic treatment (streptomycin or ampicillin) to allow our strains to colonize.

1.3 **Development of Therapeutic Bacteriophage for Sepsis:**

Bloodstream infections represent one of the most lethal forms of bacterial infections. Bacteria that gain access to the blood can spread throughout the body, and toxic products can lead to septic shock. E. coli and K. pneumoniae CRE strains are estimated to cause up to 50% mortality in these cases, owing to the severity of the condition together with resistance to antibiotics (CDC 2013). We aim to demonstrate treatment with our engineered bacteriophage therapies as alternative therapeutics to improve survival following bacterial sepsis. Furthermore, some of our engineered phages (Citorik, *Nat. Biotechnol.,* 2014) are expected to result in a decreased release of bacterial endotoxin, which may be triggered by some antibiotics or natural phages (Hagens, *Antimicrob. Agents Chemother.*, 2004).

1. **Development of Therapeutic Bacteriophage for Gastrointestinal Salmonella Infection**:

Salmonella is a major cause of morbidity worldwide, and a significant cause of mortality in children in developing countries. Oral ingestion of the bacteria leads to intestinal infection, diarrhea, and dehydration. Under this goal, we aim to develop bacteriophages capable of targeting and eliminating Salmonella and to test these therapies in vivo. Salmonella will first be administered orally to mice to establish intestinal colonization, followed by bacteriophage given orally to eliminate the bacteria. Bacterial burden will be monitored by enumerating Salmonella in the stool on selective media to assess the treatment. We aim to show improved elimination through engineering enhanced bacteriophage vehicles.

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. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OSP website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

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 (organism the sequence is from), promoter used, nature of insert (genes families are acceptable, e.g. fluorescent proteins), if 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
Phage genomes, such as members of siphoviridae, podoviridae, inoviridae, myoviridae	pRS415	native	Phage genomes	Genomic DNA	yes	100% (this is phage genome and will not infect eukaryotes)
Synthetic DNA	pRS415	native	synthetic	For mutating phage genomes	yes	0%

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

3. Do any sequences code for toxins not covered in (2) above? 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 used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, 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 strain	Reporter gene?	% viral genes	Type of promoters	Host cell
pRS415	none	Bacterial luciferase, nanoluc, lacZ, GFP, RFP	100% (phage, see note above)	PT7, PT3, PSP6, PRM, PL, PR, PBAD, Ptet, Plux, Plac, PT7lac, PT7tet	S. cerevisiae

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: https://cabescro.mit.edu/home

Viral Vector type	
Description (Plasmids used, viral vector generation for lentiviral vector)	
Source (vendor / collaborator)	
Packaging cell line(s), if applicable	
Replication competent or incompetent	
Assays for detecting replication competent virus, if applicable	
Pseudotype	
Host range	
Safety feature (e.g. self-inactivating)	
Integrate into genome (yes/no)	
Exposure hazard (e.g. insertional mutagenesis)	
Promoters to be used with viral vector	
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

3. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

a. 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 to register your animal research; If you have CAC protocol no(s), list them here: **1016-064-19, 1215-114-18**.

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?

Genetically engineered bacteriophage that show promise as antimicrobials *in vitro* will be tested in infection and disease models in mice. One current model involves skin infection caused by inoculating superficial wounds with bacteria, and treating with promising antimicrobials. A second model involves treating sepsis in mice, and a third involves manipulation of either the normal or pathological intestinal microbiome. No selection markers are engineered into the therapeutic bacteriophage.

Recipient Species	Mouse
List Pathogens, rDNA, recombinant microbe, human materials	Recombinant bacteriophage. These are not pathogens
Resulting genotype	N/A
What selection marker will be used?	None or luciferase

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. NA
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? Yes ☐ No ☒

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

1. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

2. Describe the steps used to handle and contain spills:

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/animals/plants/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 used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Acinetobacter baumannii	ATCC 19606, G7, T-40	2	ATCC, Eliava Institute	100mls		Bleach	Y
Akkermansia muciniphila	Muc [CIP 107961]	1	ATCC	100mls		Bleach	N
Citrobacter Koseri	CDC4285-83	2	SGSC	100mls		Bleach	Y
Clostridium difficile	Planned	2	ATCC, TKL	100mls		Bleach	Y
Clostridium scindens	VPI 13733	1	ATCC	100mls		Bleach	N
Clostridium spiroforme	VPI C2823-1A	2	ATCC	100mls		Bleach	Y
Clostridium symbiosum	2,M.Sebald LSU	2	ATCC	100mls		Bleach	Y
Collinsella aerofaciens	VPI 1003	1	ATCC	100mls		Bleach	N
Corynebacterium glutamicum	ATCC 13032	1	ATCC, TKL	100mls		Bleach	N
Enterobacter aerogenes	planned	2	ATCC	100mls		Bleach	Y
Enterobacter cloacae	1000654 (NDM-1)	2	ATCC	100mls		Bleach	Y
Enterococcus faecalis	Ef1 – Ef15	2	ATCC, Mia Liebermann (Fox lab)	100mls		Bleach	Y
Escherichia coli	EHEC, STEC, EPEC, the ECOR collection	2	the STEC center, ATCC	100mls		Bleach	Y
Escherichia coli K-12	MG1655, DH5alpha, EMG2, KL463, XL10, C600, DH10B, BW25113, DH5alpha Pro, MG1655 Pro, RFS289, CJ236, ER2738, ER2267, NEB10beta, E. cloni 10G, XL1-GOLD, XL1BLUE, XL1-RED, W3110, NM2, S17, CR63, CR63l, ME5486, transformax EC100D pir, Transformax EC100D pir-116, SURE2, MK01, MK02, ET12567, C600, W1, MC4100,	1	CGSC, NEB, ATCC, Thermo, Lucigen, Keith Shearwin, Laub lab, Collins lab, Agilent, expressys, invitrogen, epibio, clontech, Roberto Kolter, real biotech corporation, the Yale E. coli genetic stock center	100mls		Bleach	N

	oneshot TOP10, SHuffle-T7, MG1655(DE3), NEB10beta, MG1655 ΔntrBC, MG1655Pro with pLtetO-mf-Lon protease integrated, Transformax EPI3000, DH5alpha F' iq, sbtl3, sbtl4, stellar, HIT DH5-alpha, JM109						
Escherichia coli K-12	keio collection	1	http://cgsc.biology.yale.edu/KeioList.php	100mls		Bleach	N
Escherichia coli (synthetic)	C321.DeltaA, rEc.β.dC.12'. ΔtY	1	https://www.ncbi.nlm.nih.gov/nuccore/549811571	100mls		Bleach	N
Escherichia coli (other)	ATCC 11775, ATCC 700973, ATCC 23503, ATCC 23511, GUE, 5649, RKI, BAA-201 (TEM-3), Nissle 1917, HS, ECOR collection (ECOR1-70)	2	ATCC, P. Nordmann, STEC(http://shigatox.net/new/reference-strains/ecor.html)	100mls		Bleach	Y
Escherichia coli (Shiga-toxin producing and pathogenic)	DECA collection, EHEC 933W, EPEC, ATCC 700927, ATCC 43888, ATCC BAA-2196, ATCC BAA-2193, ATCC BAA-2193, ATCC BAA-2215, ATCC BAA-2440, ATCC BAA-2219, ATCC BAA-2192	2	ATCC, SGSC, STEC (http://shigatox.net/new/reference-strains/deca.html)	100mls		Bleach	Y
Escherichia coli B	BL21, BL21(DE3), Rosetta, Rosetta(DE3), BL21AI, BB, REL606, BL21(DE3)/pLysS, SHuffle express T7, Express Iq	1	ATCC, NEB, Lucigen, Invitrogen, TKL	100mls		Bleach	N

Escherichia coli C	C-1, C-1792 , C-236 , C-367 , C-368	1	Ian Molineux, ATCC, TKL	100mls		Bleach	N
Escherichia coli	rcF471-6, rcF479, rcF513-9	1	Mouse fecal isolates	100mls		Bleach	N
Proteus mirabilis	rcF520-22	2	Mouse fecal isolates	100mls		Bleach	Y
Eubacterium rectale	VPI 0990	1	ATCC33656	100mls		Bleach	N
Ganoderma lucidum	Planned	1	Zhao Sun, ATCC	100mls		Bleach	N
Gluconacetobacter xylinus	ATCC53582	1	ATCC	100mls		Bleach	N
Klebsiella oxytoca	**CCUG 15788**	1	Sherwood Casjens	100mls		Bleach	N
Klebsiella pneumoniae	sp. 390, MGH78578, K6 / ATCC 700603 (SHV-18), CDC1000527 (NDM-1), 1100975 (NDM-1), 1002565 (NDM-1), 1100770 (NDM-1), KPNIH1, 10, 24, 27, 29, 30, 31, 32, 33, Kp80	2	ATCC, TKL, Julie Segre, Ian Molineux, Eliava Institute	100mls		Bleach	Y
Kluyvera cryocrescens	planned	2	ATCC	100mls		Bleach	Y
Lactobacillus plantarum	WCFS1	1	ATCC	100mls		Bleach	N
lactococcus lactis	planned	1	ATCC	100mls		Bleach	N
Morganella morganii	planned	2	ATCC	100mls		Bleach	Y
Mycobacterium smegmatis (isoniazid resistance)	mc² 155	2	ATCC	100mls		Bleach	Y
Parabacteroides distastonis	mmF840, NCTC11152	2	Mouse isolate (MIT animal facility), ATCC	100mls		Bleach	Y
Prevotella copri	CB7	1	DSM18205	100mls		Bleach	N
Pseudomonas aeruginosa	PAO1, PAK, PA14, ATCC 10145, CECT111, ATCC25102, rcF477, rcF478, Ps25, Ps32	2	ATCC, Ribbeck lab, mouse fecal isolates, Eliava Institute	100mls		Bleach	Y
Pseudomonas aurantiaca	planned	1	ATCC	100mls		Bleach	N
Pseudomonas chlororaphis	planned	1	ATCC	100mls		Bleach	N
Pseudomonas citronellolis	planned	1	ATCC	100mls		Bleach	N
Pseudomonas fluorescens	A506	1	ATCC, TKL	100mls		Bleach	N
Pseudomonas putida	*KT2440, C1S, ATCC12633*	2	ATCC, TKL	100mls		Bleach	Y
Pseudomonas veronii	planned	1	ATCC	100mls		Bleach	N
Pseudomonas cellulosa	ATCC55703	1	ATCC	100mls		Bleach	N
Ruminococcus obeum	strain: VPI B321	1	ATCC29174	100mls		Bleach	N
Ruminococcus torques	strain: VPI B2-51	1	ATCC27756	100mls		Bleach	N

Pediococcus pentosaceus	DSM 20336	1	R. Rahmeh	100mls		Bleach	N
Lactobacillus brevis	ATCC 14869	1	R. Rahmeh	100mls		Bleach	N
Enterococcus faecium	JCM5804	2	R. Rahmeh	100mls		Bleach	Y
Salmonella enterica enterica Agona	SARB1	2	Salmonella enterica enterica Genetic Stock Center, ATCC, TKL	100mls		Bleach	Y
Salmonella enterica enterica Anatum	SARB2, ss44, 15/5	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica Bongori	SP141, SP905	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Brandenburg	SARB3	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Choleraesuis	SARB6, SARB7, SC-B67, 14174, A50, G9	2	SGSC, ATCC, John Elmerdahl olsen	100mls		Bleach	Y
Salmonella enterica enterica decatur	SARB8	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Derby	SARB9, SARB10	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Dublin	SARB13, SARB14, TY3627, SGSC4916, SGSC4157, 3246	2	SGSC, ATCC, John Olsen	100mls		Bleach	Y
Salmonella enterica enterica Duisberg	SARB15	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Emek	SARB20	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica enteritidis	SARB18, SARB19, LK5, ATCC 13076	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Enteritidis	Kuwait (MDR poultry isolate)	2	R. Rahmeh	100mls		Bleach	Y
Salmonella enterica enterica Gallinarum	SARB21, 287/91	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Hadar	SL485	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Heidelberg	SARA30, SARA36 to SARA40, SARB24, SL486	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Indiana	SARB25	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Kentucky	#98, SP146	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Miami	SARB26, SARB29	2	SGSC, Ian Molinzux, John Elmerdahl Olsen	100mls		Bleach	Y
Salmonella enterica enterica Montevideo	SARB31	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica münchen	SARA63 to SARA67, SARA69, SARA70, SARA72, SARB33	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Newport	SARB37	2	SGSC, ATCC	100mls		Bleach	Y

Salmonella enterica enterica Panama	SARB51, SARB52	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Reading	SARB53	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Rubislaw	SARB54	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Saint-Paul	SARA24, SARB55, SARB56	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Schwarzengrund	CVM19633	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Sendai	SARB58	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Senftenberg	SARB59	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Thompson	SARB62	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Typhimurium	LT2, IJ612, MK1046, LT1 to LT6, LT7 to LT9, A36, DT4a, LT11, LT13, LT14, LT16 to LT21, SARA1 to SARA10, SARA12, SARA24, SARB66 to SARB68, 4/74, CVM23701, M8c, TT23381, C5, SP296, SP358, SP394, SP513, SP591, SP592, SP594, SP651, SP71, SP784, SP811, SP838, SP839, SP906	2	SGSC, ATCC, John Elmerdahl olsen, Lionello Bossi, Sébastien Lemire	100mls		Bleach	Y
Salmonella enterica enterica Typhisuis	SARB69	2	SGSC, ATCC	100mls		Bleach	Y
Serratia marcescens	planned	2	ATCC	100mls		Bleach	Y
Serratia plymuthica	V4	1	ATCC, TKL	100mls		Bleach	N
Shigella boydii	ATCC 9207	2	SGSC	100mls		Bleach	Y
Shigella dysenteriae	planned	2	ATCC, Fasano lab (MGH)	100mls		Bleach	Y
Shigella flexneri	ATCC 12022, M16, 2457T	2	SGSC, ATCC	100mls		Bleach	Y
Shigella sonnei	ATCC 25931, ATCC 9290	2	SGSC, ATCC	100mls		Bleach	Y
Staphylococcus aureus	RN4220	2	L. Marraffini	100mls		Bleach	Y
Stenotrophomonas maltophilia	planned	2	ATCC	100mls		Bleach	Y
Streptococcus agalactiae	planned	2	ATCC	100mls		Bleach	Y
Streptococcus mutans	planned	1	ATCC	100mls		Bleach	N

Streptomyces coelicolor	DSMZ 41109, DSMZ 1042, DSMZ 41546	1	Kolter lab, DMSZ	100mls		Bleach	N
Vibrio cholerae	VO-258, VO-146, N16961, E7946, 569B, O395, MO10	1	Polz lab, ATCC	100mls		Bleach	N
Yersinia pseudotuberculosis	YPIII, IP2666	2	ATCC, Joan Mecsas	100mls		Bleach	Y
Yersinia enterocolityca	planned	2	ATCC	100mls		Bleach	Y
bacteriophages	T3, T4D, T6, T5, T7, lambda, P22, ES18, FelixO1, SP6, K1E, K1-5, K11, N15, K1F, FC405, KC69, RB32, RB33, RB69, Pol, Baker, K3, SboM-AG3, SnpM-CG4-1, Sens-AG11, S16, A1122, yepe2, eco32, N4, P1, P2, RB14, AC3, RB51, RB10, JSE, RB23, BBY1, D62,26, CZ, RB43, OX2, Phi1, LUZ19, GH-1, M13	1	ATCC, Ian Molineux, Tetsuro Yonesaki, Henry Krisch, lysogenic bacterial strains, natural samples	100mls		Bleach	N
saccharomyces cerevisiae	BY4741	1	TKL	100mls		Bleach	N

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

Organism	**Infectious Dose**	**Route of exposure**	**Host range**	**Clinically relevant Drug resistance**	**Availability of treatment**	**Special equipment /PPE used (above standard PPE)**	**Disease state and symptoms of exposure**
Pseudomonas (aeruginosa, putida, fluorescens)	Unknown	Ingestion, Injection/ non-intact skin	Humans	Species resistant to many antibiotics	Species susceptible to extended-spectrum penicillins (such as ticarcillin, azlocillin, and piperacillin), aminoglycosides, cephalosporins, fluoroquinolones, polymixins, and the monobactams	N/A	As opportunistic pathogens, *Pseudomonas* spp. often invades the host tissue and cause infection and bacteremia in immunocompromised hosts (e.g., HIV/AIDS, cystic fibrosis, bronchiectasis, and severe chronic obstructive pulmonary disease, burns, malignancy, or diabetes mellitus). The common site of infection is the lower respiratory tract, and severity ranges from

							colonization without immunological response to severe necrotizing bronchopneumonia; such severe infection in patients with cystic fibrosis is almost impossible to eradicate once established in the airways. Pseudomonal pneumonia often develops from oro-pharyngeal contamination or secondary bacteremia, and is also a common cause of nosocomial ventilator-related pneumonia in intensive care settings. Infections also include endocarditis, osteomyelitis, urinary tract infections, gastrointestinal infections, meningitis, and, commonly, septicaemia. *P. aeruginosa* is the most common agent associated with infection and inflammation during contact lens wear. The bacteria colonize on lenses and produce proteases to kill or invade corneal cells, an infection that can lead to scarring and vision loss. The species is also the most virulent with a mortality rate of 30%, which can be higher depending on predisposing conditions. *P. aeruginosa* can also readily colonize on open burn wounds, causing infections, abscesses, and sepsis, with edema and/or discoloration of unburned skin at wound margins and green pigment in subcutaneous fat. *P. aeruginosa* is also associated with swimmer's ear (otitis externa). Other *Pseudomonas* species are also opportunistic;

							however, cases of infection are rare.
Clostridium symbiosum, C. spiroforme		Injection/ non-intact skin	Humans		most species are susceptible to penicillin, clindamycin, chloramphenicol, piperacillin, metronidazole, imipenem, and combinations of b-lactams with b-lactamase inhibitors.		Illnesses primarily associated with Clostridium spp. are: Clostridial bacteremia: Symptoms can vary greatly but will typically include fever, chills, and leukocytosis. The fatality rate ranges from 25-50%. Many Clostridium spp. can be associated with anaerobic bacteremia including C. septicum, C. ramosum, C. clostridioforme, or C. tertium
ENTEROCOCCUS FAECALIS	Unknown	Ingestion; mucosal contact; injection/ non-intact skin	Humans	Strains resistant to β-lactams, aminoglycosides and, increasingly, vancomycin have been described[2,4]. Strains have also been identified which carry genetic elements conferring resistance to chloramphenicol, tetracyclines, macrolides, lincosamides, quinolones, and streptogramins[2].	Most strains remain susceptible to penicillin, ampicillin, and vancomycin.		Enterococci can cause urinary tract, wound, and soft tissue infections[2,4]. They are also associated with bacteremia which can lead to endocarditis in previously damaged cardiac valves[4]. E. faecalis is the most frequent species isolated from human intestine samples (80-90%), E. faecium accounts for 5-10% of isolates[1
Kluyvera cryocrescens	Unknown	Injection/ non-intact skin	Humans	Unknown	Antimicrobial agents active against most Kluyvera strains include third-generation cephalosporins, fluoroquinolones, and aminoglycosides	N/A	soft tissue infection; wound infections; site infections Kluyvera rarely causes disease in humans. West et al. 1998 Diagnostic Microbiology and Infectious Disease 32:237-241
Morganella morganii	Unknown	Injection/ non-intact skin	Humans	M. morganii strains are resistant to penicillin, ampicillin/sulbactam, oxacillin, first-generation and second-generation cephalosporins, macrolides, lincosamides, fosfomycin, colistin, and polymyxin B.[3] The emergence of highly resistant strains of M. morganii have been associated with use of third-generation cephalosporins.	○ Treatment of M. morganii infections may include: Ticarcillin, Piperacillin, Ciprofloxacin, Third-generation and Fourth-generation cephalosporins,		There have been several reports that M. morganii causes sepsis, ecthyma, endophthalmitis, chorioamnionitis, however more commonly urinary tract infections, soft tissue infections, septic arthritis, meningitis and bacteremia often with fatal consequences. Polymicrobial infections are most abundantly caused by this microbe which additionally damages

							the skin, soft tissues, and urogenital tract can be cured through use of the aforementioned antibiotics.
Salmonella enterica (non typhoidal)	For non-typhoidal salmonellosis, the infectious dose is approximately 10^3 bacilli (4, 7). For enteric fever, the infectious dose is about 10^5 bacilli by ingestion (4, 6, 7). Patients with achlorhydria, depressed cell-mediated immunity, or who are elderly may become infected with at a lower infectious dose (4, 7). The infectious dose may also be dependent on the level of acidity in the patient's stomach	Ingestion; Injection/ non-intact skin	Humans	Some resistance to chloramphenicol has been reported and, in 1989, 32% of strains were multi-drug resistant	Susceptible to chloramphenicol, ciproflaxin, amoxicillin, co-trimoxazole, trimethprim-sulfonamid, cephalosporins and norfloxacin		Salmonella enterica can cause four different clinical manifestations: gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state (7)
Stenotrophomonas maltophilia	Unknown	Ingestion; mucosal contact; injection/ non-intact skin	Humans	Species is not usually susceptible to piperacillin, and susceptibility to ceftazidime is variable	Many strains of S. maltophilia are sensitive to co-trimoxazole and ticarcillin,	N/A	S maltophilia has few pathogenic mechanisms and, for this reason, predominantly results in colonization rather than infection. If infection does occur, invasive medical devices are usually the vehicles through which the organism bypasses normal host defenses. Otherwise, the pathophysiology of this nonfermentative aerobic gram-negative bacillus does not differ from other nonfermentative aerobic organisms
Streptococcus agalactiae	Unknown	Mucosal contact; Injection/ non-intact skin	Humans	some strains penicillin tolerant and require treatment with an	Sensitive to penicillin or ampicillin		It presents with nonspecific symptoms, such as fever, vomiting and irritability, and can consequently lead to

				aminoglycoside as well.			late diagnosis. Hearing loss can be a long-term sequela of group B Streptococcus species (GBS)-meningitis. Infection with GBS is the cause of some instances of stillbirth.
Vibrio cholerae	106-1011 organisms	Ingestion; injection/ non-intact skin	Humans	An outbreak in 1979 in Bangladesh was caused by multi-drug resistant strains of El Tor biotype. 36% of strains in this outbreak were resistant to tetracycline, ampicillin, kanamycine, streptomycin, and trimethoprim sulfamethoxazole. Resistance has been shown to nalidixic acid, furazolidone, and co-trimoxazole, V. cholerae O1 Inaba isolates have been found to be multi-antibiotic resistant, when increasing resistance to ciprofloxacin	Tetracycline has been the drug of choice, although resistance to this antibiotic is becoming more common. Ciproflaxin, doxycycline and co-trimoxazole can also be used		Vibrio cholerae can cause syndromes ranging from asymptomatic to cholera gravis. In endemic areas, 75% of cases are asymptomatic, 20% are mild to moderate, and 2-5% are severe forms like cholera gravis. Symptoms include abrupt onset of watery diarrhoea (a grey and cloudy liquid), occasional vomiting and abdominal cramps. Dehydration ensues with symptoms and signs such as thirst, dry mucous membranes, decreased skin turgor, sunken eyes, hypotension, weak or absent radial pulse, tachycardia, tachypnea, hoarse voice, oliguria, cramps, renal failure, seizures, somnolence, coma and death. Death due to dehydration can occur in hours to days in untreated children and the disease is dangerous for pregnant women and their foetuses during late pregnancy as abortion, premature labor and fetal death may occur. In cases of cholera gravis involving severe dehydration, up to 60% of patients can die; however, less than 1% of cases treated with rehydration therapy are fatal. The disease typically lasts from 4-6 days. Worldwide, diarrhoeal disease, caused by cholera and many other pathogens, is the second leading cause of death for children under the age of 5 and

							at least 120,000 deaths are estimated to be caused by cholera each year. In 2002, the WHO deemed that the case fatality ratio for cholera was about 3.95%.
Acinetobacter baumannii	Unknown	Inhalation ; injection/ non-intact skin	Humans	Uknown	Meropenem, colistin, polymyxin B; amikacin, rifampin, minocycline, tigecycline		Ranging from pneumonia to serious blood or wound infections, and the symptoms vary depending on the disease. *Acinetobacter* may also “colonize” or live in a patient without causing infection or symptoms, especially in tracheostomy sites or open wounds.
Klebsiella pneumoniae	Unknown; according to one source may be 10^8 Klebsiella organisms per gram of feces are required to produce damage	Ingestion; mucosal contact; inhalation ; injection/ non-intact skin	Humans	Since more and more strains of Klebsiella spp. appear to be developing and harbouring extended-spectrum beta-lactamases (ESBLs), cephalosporinases, and carbapenemases, resistance of Klebsiella spp. to current antibiotics appears to be increasing these strains are not pan-resistant to all beta-lactam antibiotics, but have differing susceptibility protocols, so that in some cases beta-lactams can still be used to treat them, although in high concentration. Other treatment regimes include beta-lactams in combination with a beta-lacatmase inhibitor, tigecycline, colistin and aztreonam	Klebsiella spp. are known to show resistance to penicillins, especially ampicillin and carbenicillin Other treatment regimes include beta-lactams in combination with a beta-lactamase inhibitor, tigecycline, aztreonam, and colistin. According to results from some studies in Europe and USA, ranges of susceptibility were as follows [1]: ceftazidime (92-95%), ceftriaxone (96-98%), cefotaxime (96%), piperacillin-tazobactam (90-97%), imipeneum (98-100%), gentamicin (95-96%), amikacin (98-99%), triethoprimsulfamet hoxazole (SXT) (88-90%).		Respiratory Disease: K. pneumoniae – a leading cause of community-acquired and nosocomial pneumonia and lung abscesses. Infection of the upper lobe is more common. Symptoms include: fevers, chills, and leukocytosis with red currant jelly-like sputum [1]. Rare complications include lung infection involving necrosis and sloughing of the entire lobe. Central nervous system (CNS) infections: K. pneumoniae – cause community-acquired meningitis and brain abscesses. Clinical symptoms include: headaches, fever, altered conciousness, seizures, and septic shock. Hepatic disease: K. pneumoniae – an important causative pathogen for pyogenic liver abscesses with symptoms including fever, right-upper-quadrant pain, nausea, vomiting, diarrhoea or abdominal pain, and leukocytosis. Abscesses occur predominantly in the right lobe and are solitary

Shigella flexneri, dysenteriae and boydii	10-200 organisms	Ingestion; mucosal contact; inhalation; injections /non-intact skin	Humans	Multidrug-resistant strains are emerging, including those against trimethoprim-sulfamethoxazole (TMP-SMX), ampicillin, and chloramphenicol	Susceptible to ampicillin, trimethoprim, sulfamethoxazole, naldixic acid, ofloxacin, chloramphenicol, fluoroquinolones, and ciprofloxacin		Infection may be mild and asymptomatic, but it is most commonly characterized by acute intestinal infections upon ingestion, resulting in mild watery diarrhea to severe inflammatory bacillary dysentery or shigellosis, manifested by severe abdominal cramps, nausea and vomiting, fever, tenesmus, anorexia, and stool containing blood and mucus [1, 2, 8]. Further complications include Reiter's syndrome which has been associated with S. flexneri [9, 10], severe dehydration, intestinal perforation, toxic mega colon, bacteremia, toxaemia [11], septicaemia, seizures, toxic encephalopathy with headache and alterations of consciousness, septic shock and convulsions (very rare) [4], and haemolytic uremic syndrome, which have been linked to Shiga toxin (a potent cytotoxin produced by S. dysenteriae that can also cause other neurotoxic effects). Virulence of Shigella is temperature-regulated, as organisms are able to invade HeLa cells at 37°C, and cannot do so in vitro at 30°C [12]. Infections are usually self-limiting, but can become life-threatening in immunocompromised patients or if not properly treated. Severity of infection depends on the host, dose, and serotype [2]. S. dysenteriae is the most pathogenic species, with a fatality rate up to 20%, whereas S. sonnei usually cause mild forms of shigellosis.

E. coli other than laboratory strains, EHEC, STEC, EPEC, the ECOR collection	Ranges from 10-100 for EHEC strains to 10^6-10^8 for other strains	Ingestion; mucosal contact; inhalation; injection/ non-intact skin	Humans; cattle, all animals	Some of these strains are carbapenem-resistant, but not pan-resistant to antibiotics.	Therapeutic treatment E coli meningitis requires antibiotics, such as third-generation cephalosporins (eg, ceftriaxone). E coli pneumonia requires respiratory support, adequate oxygenation, and antibiotics, such as third-generation cephalosporins or fluoroquinolones. E coli cholecystitis/cholangitis requires antibiotics such as third-generation cephalosporins that cover E coli and Klebsiella organisms. Empiric coverage should also include anti– E faecalis coverage. For E coli intra-abdominal abscess, antibiotics also must include anaerobic coverage (eg, ampicillin and sulbactam or cefoxitin). In severe infection, piperacillin and tazobactam, imipenem and cilastatin, or meropenem may be used. Combination therapy with antibiotics that cover E coli plus an antianaerobe can also be used (eg, levofloxacin plus clindamycin or metronidazole). E coli enteric infections require fluid replacement with solutions containing appropriate electrolytes. Antimicrobials known to be useful in cases of traveler's diarrhea include doxycycline, trimethoprim/sulfamethoxazole (TMP/SMZ),		Beneficial strains of E. coli typically colonize the infant gastrointestinal tract within a few hours after birth. The presence of this bacterial population in the intestine suppresses the growth of harmful bacteria and is important for synthesizing appreciable amounts of B vitamins. E. coli usually remains harmless when confined to the intestinal lumen. However, in debilitated or immuno-suppressed humans, or when gastrointestinal barriers are violated, even normal, "non-pathogenic" strains of E. coli can cause infection. It is also known that some E. coli strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system in even very healthy people. Strains of E. coli that cause diarrhea include strains that cause traveler's diarrhea (enterotoxigenic E. coli), persistent diarrhea (enteroaggregative E. coli), watery diarrhea of infants (enteropathogenic E. coli), hemorrhagic colitis (bloody diarrhea), and hemolytic uremic syndrome (enterohemorrhagic E. coli) Shiga toxin-producing E. coli (STEC) can cause the following symptoms: Nausea, Severe abdominal cramps, Watery or very bloody diarrhea, Fatigue. STEC can also cause low-grade fever or

					fluoroquinolones, and rifaximin. They shorten the duration of diarrhea by 24-36 h. Antibiotics are not useful in enterohemorrhagic E coli (EHEC) infection and may predispose to development of HUS. Antimotility agents are contraindicated in children and in persons with enteroinvasive E coli (EIEC) infection. Uncomplicated E coli cystitis can be treated with a single dose of antibiotic or 3-d course of a fluoroquinolone, TMP/SMZ, or nitrofurantoin. Recurrent E coli cystitis (ie, >2 episodes/y) is treated with continuous or postcoital prophylaxis with a fluoroquinolone, TMP/SMZ, or nitrofurantoin. Patients with complex cases (eg, those with diabetes, >65 y, or recent history of UTI) are treated with a 7- to 14-d course of antibiotics (eg, levofloxacin, third-generation cephalosporins, or aztreonam). Acute uncomplicated E coli pyelonephritis in young women is treated with fluoroquinolone or TMP/SMZ for 14 d. Patients with vomiting, nausea, or underlying illness (eg, diabetes) should be admitted to the hospital. If fever and flank pain persist for more than 72 h, ultrasonography or CT scanning may be performed.		vomiting. Symptoms usually begin from 2 to 5 days after eating contaminated food or drinking contaminated liquids. Symptoms may last for 8 days, and most people recover completely from the disease.

					Treat E coli perinephric abscess or prostatitis with at least 6 wk of antibiotics. E coli sepsis requires at least 2 wk of antibiotics and identification of the source of bacteremia based on imaging study results. McGannon et al found that antibiotics that target DNA synthesis, such as ciprofloxacin (CIP) and TMP/SMZ, showed increased Shiga toxin production, whereas antibiotics that target the cell wall, transcription, or translation did not. Remarkably, high levels of Shiga toxin were detected even when growth of O157:H7 was completely suppressed by CIP. In contrast, azithromycin significantly reduced Shiga toxin levels even when O157:H7 viability remained high. Extended-spectrum cephalosporins are widely administered to treat serious infections due to gram-negative bacilli. However, managing infections due to ESBL-producing isolates is particularly challenging since these isolates have varying levels of resistance to agents in the extended-spectrum cephalosporin class, in addition to being multiply resistant to other antimicrobials such as aminoglycosides, sulfonamides, and fluoroquinolones		

					A MEDLINE search was performed for all studies published in the English literature using key words "ESBL" and "extended-spectrum ß-lactamase." Studies that provided treatment information on patients infected with a putative or known ESBL-producer were evaluated. An isolate tested positive with the double-disk synergy test and/or Etest strips (AB Biodisk) was considered a putative ESBL producer unless molecular analysis was performed to characterize the enzyme type(s).		
YERSINIA PSEUDOTUBERCULOSIS	10^8 bacteria or more orally	Ingestion; injection/ non-intact skin	Humans; rabbits, rodents, cattle, pigs, pets, wild mammal and birds	Unknown	Susceptible to ampicillin, third generation cephalosporins, aminoglycosides, tetracyclines, and chlorampheinicol.		Yersinia pseudotuberculosis is a rare cause of acute enteric disease with symptoms such as acute mesenteric lymphadenitis and gastroenteritis associated with abdominal pain and fever (diarrhea is unusual). One to 3 weeks after the acute phase of the disease, post-infectious complications can occur, such as reactive arthritis and erythema nodosum. The arthritic phase of the disease can last up to 6 months. Other complications include lesions to lymph nodes, spleen and liver, as well as septicaemia in immunocompromised patients. The disease is most common in children and young adults and immunocompromised individuals are at greater risk of severe disease or death.

YERSINIA ENTEROCOLITICA	10^8 bacteria or more orally	Ingestion; injection/ non-intact skin	Humans; farm animals, birds, pets	It is generally resistant to penicillin and its derivatives and to narrow spectrum cephalosporins	Yersinia enterocolitica is susceptible to chloramphenicol, fluoroquinolones, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole.		Yersinia enterocolitica infection is characterized by enteritis, enterocolitis (particularly in children), fever (39ºC), watery stools, abdominal pain and acute mesenteric lymphadenitis (which may mimic appendicitis). In some cases acute terminal ileitis and enteric fever can occur. 1-3 weeks after the initial clinical symptoms, reactive arthritis and erythema nodosum may occur which can last about 6 months after infection. In rare instances, complications can include meningitis, endophthalmitis, conjunctivitis, myocarditis, pneumonia, pulmonary abscess, hepatitis, cholangitis, peritonitis, glomerulonephritis, urethritis, cellulitis, haemolytic anaemia, thyroiditis, pharyngitis and septicaemia
Clostridium difficile	Unknown	Ingestion	Humans, pigs, calves	Some rare strains are resistant to metronidazole; Fluroquinolone-resistant hypervirulent strain 027 isolates in US	Susceptible to metronidazole, oral vancomycins; penicllins and cephalosporins in vitro		C. difficile is the main cause of nosocomial antibiotic- associated diarrhea. Antibiotics or any other procedures that disrupt the normal intestinal microbiota can lead to the overgrowth and production of toxin by C. difficile, which leads to clinical manifestations of infection. The diarrhea may range from a few days of intestinal fluid loss to life-threatening pseudomembranous colitis. In diarrhea without pseudomembranous colitis (PMC), feces have a foul odor and are not bloody. Abdominal pain with or without pyrexia may also be present along with diarrhea. PMC is associated with intense inflammation of the colon and

							formation of pseudomembranes on the intestinal mucosal surface. Patients with PMC also have more systemic side effects.
ENTEROBACTER AEROGENES and cloacae	Unknown	Ingestion; injection/ non-intact skin	Humans	Enterobacter spp. are resistant to ampicillin; first- and second- generation cephalosporins[7]; and cephalothin(	Most Enterobacter spp. are susceptible to cefepime[7], aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole[8]. Tigecycline has been shown effective in vitro		Enterobacter spp., particularly E. aerogenes and E. cloacae, have been associated with nosocomial outbreaks, and are considered opportunistic pathogens[1,5]. Enterobacter spp. can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections[6,7]. In addition, Enterobacter spp. have been noted in intravascular device-related infections, and surgical site infections (primarily postoperative or related to devices such as biliary stents)[7]. Many species can cause extra-intestinal infections[6], for example, Enterobacter sakazakii, has been associated with brain abscesses in infants and with meningitis[3,7]. Mortality rates for bacterial meningitis range from 40-80%[5].
SERRATIA MARCESCENS and PLYMUTHICA	Unknown	Injection/ non-intact skin	Humans	Many Serratia spp. isolates (39-73%) are resistant to gentamicin. They are all resistant to penicillins and cephalosporin	Serratia spp. are usually susceptible to aminoglycosides, fluoroquinolones, and co-trimazole		Serratia spp. are opportunistic pathogens and are one of the ten most common causes of bacteremia in North America. They are responsible for a variety of infections, including bacteremia, pneumonia, intravenous catheter-associated infections, osteomyelitis, endocarditis, and, rarely, endogenous and exogenous endophthalmitis. Symptom of

							endophthalmitis appears rapidly after infection, and may include fever, erythema, ocular pain, periorbital swelling, and hypopyon (pus in the eyes). The mortality rate from bacteremia due to Serratia spp. 6 months after infection is 37%.

a2. List and describe any other biological material not covered in the tables above:

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

1. Briefly describe experiment and procedures involving use of biological agents?

Bacterial growth is done following standard protocols as described in Current Protocols in Molecular Biology[2]. Phage transfection, titering, and purification are performed by standard protocols, as described in https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975003/

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:

Engineered bacteriophage will have altered host range to improve antimicrobial activity, or act against new pathogens.

3. Will you introduce viable biological material (microorganisms or cells) into animals?

Yes ☒ No ☐ If so:

a) Please describe:

Genetically engineered bacteriophage that show promise as antimicrobials *in vitro* will be tested in infection and disease models in mice. One current model involves skin infection caused by inoculating superficial wounds with bacteria, and treating with promising antimicrobials. A second model involves treating sepsis in mice, and a third involves manipulation of either the normal or pathological intestinal microbiome. No selection markers are engineered into the therapeutic bacteriophage.

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:__**1016-064-19, 1215-114-18**__________

Project 2: Bacteria as a tool for Manufacturing and Testing Bioactive Molecules

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

As part of their physiology, bacteria manufacture a vast number of enzymes, small molecules, and polymers, which alone and in groups perform complex tasks. In these projects we are modifying bacteria to make them make novel products such as therapeutic proteins, small molecule and protein antibiotics. We are testing antibiotics, both those produced by engineered bacteria and those produced synthetically. In addition, we are developing systems to increase production of chosen products, to allow for efficient manufacture and purification of valuable bioactive molecules.

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.

2.1) Synthetic microbial consortia for the production of value added small molecules and therapeutic applications

Our goal is to create a broad, multidisciplinary set of technologies to achieve robustness, stability, and safety in multi-species communities that perform collaborative functions. Currently, the dynamic mechanisms governing multi-species relationships are poorly defined. Moreover, current microbial engineering revolves around individual and highly domesticated bacteria, living in highly constrained environments, devoid of fluctuations and competitors. These organisms that are susceptible to mutations, synthetic gene constructs that impose significant loads on their hosts and are not optimized for stable and robust function, the bacteria share the standard genetic code and are thus non-orthogonal with other genetic elements and wild organisms, and the systems that are incapable of sensing malfunctions and triggering organismal and functional termination with high efficiencies. The resulting lack of robustness, stability, and safety make the use of such engineered organisms in the real world infeasible. We will create, model, and study the properties and design rules that control the behavior of multi-species microbial consortia.

One specific goal is to develop an engineered 5-species microorganism consortium for inflammatory bowel disease (IBD) therapy. More than 1 million people in the US suffer from IBD, and the cause and cure are not known. There is evidence that intestinal microbiota act as autoantigens in IBD; there are also changes in the population as a result of the disease. Our goal is to use a microbial consortium to decrease inflammation in the colon, with the hope that this will both ease symptoms, and allow the intestine to heal.

The five species we are going to engineer are *Bacteroides Fragilis*; *Bacteroides thetaiotaomicron; Bacteroides ovatus; Bacteroides vulgatus* and *Escherichia coli* Nissle. Each microorganism will express one or two enzymes from this enzyme list: Superoxide dismutase; Catalase; Glutathione peroxidase; Glutathione Reductase; Nitric oxide dioxygenase; Peroxiredoxin; Disulfide Reductase. The enzymes will work together to detoxify reactive oxygen species and reactive nitrogen species implicated as toxins in IBD.

We will also investigate conditions for creating consortia including other bacteria, such as *Enterobacter aerogenes, Pseudomonas aurantiaca, Pseudomonas chlororaphis, Pseudomonas citronellolis, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas veronii, Serratia marcescens, Bacillus subtilis 168,* and *Lactobacillus plantarum*. These experiment do not involve genetic engineering of the bacteria at this time.

2.4) Use of CRISPRi to engineer the production of amino-acids by Corynebacterium glutamicum

In this project, sgRNAs were designed to be co-expressed with a nuclease-deficient Cas9 (dCas9). In this system, the transcription of genes will be repressed; each of several genes can be repressed independently. The amount of glutamate or lysine secreted by the organism was then determined. CRISPRi can be successfully used to the control of metabolic pathways in *C. glutamicum*. This will allow the increased production of economically relevant bioproducts.

2.5) Therapeutic use of anti-microbial peptides

Antibiotic resistance is a serious global health problem. Increasing numbers of bacterial isolates are resistant to all available antibiotics, and the untreatable infections they cause are predicted to kill 10 million people per year worldwide by 2050. Therefore, there is an urgent need to develop alternative approaches, extending beyond conventional antibiotics, to treat severe bacterial infections. The innate immune systems of all known multicellular organisms, including humans, produce evolutionarily conserved small molecules known as antimicrobial peptides (AMPs). These naturally occurring peptides, which have evolved over billions of years, confer protection against a wide range of pathogenic microorganisms. They therefore constitute excellent templates for the generation of engineered peptides having enhanced activity against highly drug-resistant bacterial infections.

The experimental procedures will involve treating different bacterial species, including drug-resistant pathogens, with a newly designed and synthesized library of antimicrobial peptides. The model organism *Escherichia coli*, which lives in the lower intestine of warm-blooded organisms, will be used as proof-of-concept and results obtained with this organism will be extended to more relevant clinical antibiotic-resistant strains available in the Lu lab (see section 1). We will also cross-check results other model pathogenic organisms such as, *Salmonella, Klebsiella*, *Shigella*, pathogenic and non-pathogenic *Escherichia coli*, *Pseudomonas*, *Yersinia*, *Enterobacter, Enterococcus,* and *Clostridium difficile*.

We have recently observed that some synthetic peptides are capable of killing bacterial species from the human microbiota. This is interesting because these strains are known to be completely resistant to killing by naturally occurring AMPs. Therefore, these synthetic peptides could serve as tools to engineer the microbiome by either 1) selectively removing specific species or 2) indiscriminately killing all species in a particular microbial consortium.

Therefore, we will expand our screen by challenging additional members of the human microbiota. All of the strains that will be tested are natural members of a healthy human microbiota. They include *Bacteroides* strains that have been previously used extensively in the lab and can potentially rarely cause anaerobic infections in immunocompromised individuals. *Bacteroides spp.* are therefore listed as BL2. All of the other strains are listed as BL1 and are non-pathogenic. We will use only wild-type strains for the proposed experiments and, therefore, these strains will not be genetically modified.

2.6) Probiotic *E. coli* for the *in situ* production of anti-inflammatory compounds

Inflammation in the gut is linked to a growing number of medical conditions including but not limited to IBD, Crohn's disease or cancer. With this project, we are exploring the possibility to create commensal bacteria that may lower inflammation in situ by producing anti-inflammatory compounds such as anti-TNF-α, IL-10 and anti-IL-23. We will introduce the pathways for synthesis and export of such molecules into the common laboratory *E. coli* strain BL21(DE3) for initial testing and then transfer the entire system into the probiotic *E. coli* Nissle 1917 for in animal testing. The pathways will be partly integrated into the genome of our final strain and partly carried on replicative plasmids (Psang10-3F, pTRKH3-ermGFP, pGEN-luxCDABE, pTKW106alp7A) chosen according to our guidelines and sourced from Addgene. Eventually, we hope to create a strain which is plasmid free and antibiotic resistance marker free. After having ascertained proper expression and export of our effectors *in vitro*, and exact bacterial loads determined, the strains will be gavaged to TNBS treated mice. TNBS is a chemical that induces a Crohn's disease-like state in mice. We rely on *E. coli* Nissle 1917 because it was shown to be either neutral or beneficial in several infectious contexts. Genetically modifying it is easy and there is no reason why any of the modification we will incur to it may make it more pathogenic.

2.7) Identification of genetic targets leading to antibiotic resensitizing.

Many bacteria are naturally resistant to a variable array of antibiotics. This is often achieved through expression of powerful molecular pumps that expel antibiotics but can also be the result of alterations of the bacterial membranes or surface appendages, mutation of the antibiotic target or from the formation of biofilms. The goal of this project is to resensitize *Pseudomonas aeruginosa* to standard antibiotics by reducing expression of biofilm genes, and prevent biofilm formation. *Pseudomonas aeruginosa* is a common cause of healthcare-associated infections and, in the United States, more than 13% of these infections are associated with multidrug-resistant phenotypes. These bacteria form protected communities known as biofilms and they can be up to 1000-fold more resistant to antibiotics. Therefore, strategies to identify and validate new targets that resensitize *P. aeruginosa* to antibiotics are needed. The first step is using CRISPR interference to achieve transcriptional repression of selected

target genes (*flgK*, *phzM*, *pslA*, *pslB*, *relA* and *lasI* that are known to be involved in antibiotic resistance and/or biofilm synthesis). Antibiotic resistance of the resulting strains will then be assessed in Minimum Inhibitory Concentration and growth rate with/without antibiotic experiments conducted on biofilms grown in a microfluidic device.

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. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OSP website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

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:

5. Specify DNA/RNA source (organism the sequence is from), promoter used, nature of insert (genes families are acceptable, e.g. fluorescent proteins), if 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
CRISPR/cas system components	Vectors from the Lutz and Bujard collection[1], pNBU1, pNBU2, pZA1, pZE1, pZE2, pZE3, pBR322, pAL374 and pZ8−1, pBR322:RSF1010	constitutive	S. pyogenes	CRISPR-Component	Yes	0
Enzymes such as Superoxide dismutase; Catalase; Glutathione peroxidase; Glutathione Reductase; Nitric oxide dioxygenase; Peroxiredoxin; Disulfide Reductase	pNBU1, pNBU2, pZA1, pZE1, pZE2, pZE3, pBR322	constitutive	E coli	Enzymes that break down reactive oxygen and reactive nitrogen	yes	0
Anti-inflammatory compounds such as anti-TNF-α, IL-10 and anti-IL-23	pET28 family	T7 promoter	E coli BL21DE	Anti-inflammatory compounds	yes	0

Anti-inflammatory compounds such as anti-TNF-α, IL-10 and anti-IL-23	Psang10-3F, pTRKH3-ermGFP, pGEN-luxCDABE,	constitutive	E coli Nissle 1917	Anti-inflammatory compounds	yes	0

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

7. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

8. 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:

3. Identify cloning/expression/transfection vectors used, recipient bacterial strains used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, 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 strain	Reporter gene?	% viral genes	Type of promoters	Host cell
Vectors from the Lutz and Bujard collection[1].	E coli K12, MG1655	fluorescent proteins, as described above	0	Constitutive, inducible such as pOxyR, pSoxS, pLux, pLas, pRhl (described in detail above	E coli K12 or MG1655
pNBU1, pNBU2	Bacteroides thetaiotaomicron, B. fragilis, B. ovatus, B. vulgatus	Y	0	Constitutive and inducible	Bacteroides thetaiotaomicron, B. fragilis, B. ovatus, B. vulgatus
pZA1	E. coli Nissle 1917	N	0	Constitutive	E. coli Nissle 1917
pZE1, pZE2, pZE3	E. coli Nissle 1917	N	0	Constitutive	E. coli Nissle 1917
pBR322	E coli K12	Y	0	Constitutive	E. coli K12
pAL374 and pZ8−1	*Corynebacterium glutamicum*	N	0	constitutive	*Corynebacterium glutamicum*
pBR322:RSF1010	E coli K12 and Pseudomonas aeruginosa	N	0	constitutive	Pseudomonas aeruginosa
pET28 a, b, c	E coli BL21 DE	N	<10%	T7 promoter	E coli BL21 DE
Psang10-3F, pTRKH3-ermGFP, pGEN-	E coli Nissle 1917	N	0	costitutive	E coli Nissle 1917

luxCDABE, pTKW106alp7A					

4. 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: https://cabescro.mit.edu/home

Viral Vector type	
Description (Plasmids used, viral vector generation for lentiviral vector)	
Packaging cell line(s), if applicable	
Replication competent or incompetent	
Assays for detecting replication competent virus, if applicable	
Pseudotype	
Host range	
Safety feature (e.g. self-inactivating)	
Integrate into genome (yes/no)	
Exposure hazard (e.g. insertional mutagenesis)	
Promoters to be used with viral vector	
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

4. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

a. 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 to register your animal research; If you have CAC protocol no(s), list them here:_ **0915-092-18; 1215-114-18, 1016-064-19.**
_. The questions below are intended to deal with the use of potentially biohazardous agents in animals/insects or the creation of transgenic animals/insects.

2. 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?

0915-092-18:

We will introduce recombinant organisms, including engineered E. coli, B. thetaiotaomicron, B. fragilis, B. ovatus and B. vulgatus strains, as well as the probiotic yeast S. boulardii into the mouse gastrointestinal tract. The objective is to test genetically engineered strains for their ability to detect gut inflammation and/or secrete anti-inflammatory proteins, for their ability to act as as next-generation of inflammation bowel disease sensors and therapeutics. Organisms will be introduced by oral gavage. Recipient species includes Balb/c mice, SJL mice, and C57BL/6 mice. No engineered bacterial strains are known to have or will acquire virulence. The bacteria may have display resistance to common laboratory antibiotics such as kanamycin, or erythromycin, but not to clinically relevant drugs.

1215-114-18:
Development of Therapeutic Bacteriophage for Decolonization: A major concern in infectious disease is the establishment of antibiotic resistance and pathogen reservoirs in healthy individuals that could potentially later cause life threatening opportunistic infections. We wish to explore the use of bacteriophage as a means to specifically eliminate antibiotic resistant or pathogenic strains from a microbiome without affecting commensal populations. We intend first demonstrate this concept using non-pathogenic *E. coli* strains (K-12, BL21, C-1) and *Klebsiella spp.* (sp. 390 and *oxytoca*). Later, we extend this concept to clinical isolates carbapenem-resistant Enterobacteriaceae (CREs), including *E. coli* and *K. pneumoniae* strains. As many of these human isolates do not naturally colonize the mouse gut, we will perturb the microbiota using antibiotic treatment (streptomycin or ampicillin) to allow our strains to colonize.

1016-064-19
The goal is determine whether different agents such as ionic liquids, nitroxides and antimicrobial peptides exhibit anti-infective properties *in vivo* in two skin infection models, superficial and abscess. There is a need for alternative therapeutics to treat drug- resistant infections. Here, we aim to test the efficacy of novel classes of antimicrobials in mouse models

4. 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.

5. 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? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

3. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

4. Describe the steps used to handle and contain spills:

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/animals/plants/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 used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Bacillus subtilis	168, NCIB3610	1	Kolter lab, TKL	100mls		Bleach	N
Bacteroides fragilis	NCTC 9343	2	ATCC	100mls		Bleach	Y
Bacteroides ovatus	NCTC 11153, ATCC 8483	2	ATCC	100mls		Bleach	Y
Bacteroides thetaiotaomicron	VPI-5482, NCTC 9343, HSP40	2	ATCC	100mls		Bleach	Y
Bacteroides uniformis	ATCC 8492	2	ATCC	100mls		Bleach	Y
Bacteroides vulgatus	ATCC 8482, mmF837	2	ATCC, mouse isolate MIT	100mls		Bleach	Y
Bacteroides vulgatus	ATCC 8482	2	ATCC	100mls		Bleach	Y
Corynebacterium glutamicum	ATCC 13032	1	ATCC, TKL	100mls		Bleach	N
Enterobacter aerogenes	planned	2	ATCC	100mls		Bleach	Y
Enterobacter cloacae	1000654 (NDM-1)	2	ATCC	100mls		Bleach	Y
Enterococcus faecalis	Ef1 – Ef15	2	ATCC, Mia Liebermann (Fox lab)	100mls		Bleach	Y
Escherichia coli	EHEC, STEC, EPEC, the ECOR collection	2	the STEC center, ATCC	100mls		Bleach	Y
Escherichia coli K-12	MG1655, DH5alpha, EMG2, KL463, XL10, C600, DH10B, BW25113, DH5alpha Pro, MG1655 Pro, RFS289, CJ236, ER2738, ER2267, NEB10beta, E. cloni 10G, XL1-GOLD, XL1BLUE, XL1-RED, W3110, NM2, S17, CR63, CR63l, ME5486, transformax EC100D pir, Transformax EC100D pir-116, SURE2, MK01, MK02, ET12567, C600, W1, MC4100, oneshot TOP10, SHuffle-T7,	1	CGSC, NEB, ATCC, Thermo, Lucigen, Keith Shearwin, Laub lab, Collins lab, Agilent, expressys, invitrogen, epibio, clontech, Roberto Kolter, real biotech corporation, the Yale E. coli genetic stock center	100mls		Bleach	N

	MG1655(DE3), NEB10beta, MG1655 ΔntrBC, MG1655Pro with pLtetO-mf-Lon protease integrated, Transformax EPI3000, DH5alpha F' iq, sbtl3, sbtl4, stellar, HIT DH5-alpha, JM109						
Escherichia coli K-12	keio collection	1	http://cgsc.biology.yale.edu/KeioList.php	100mls		Bleach	N
Escherichia coli (synthetic)	C321.DeltaA, rEc.β.dC.12'. ΔtY	1	https://www.ncbi.nlm.nih.gov/nuccore/549811571	100mls		Bleach	N
Escherichia coli (other)	ATCC 11775, ATCC 700973, ATCC 23503, ATCC 23511, GUE, 5649, RKI, BAA-201 (TEM-3), Nissle 1917, HS, ECOR collection (ECOR1-70)	2	ATCC, P. Nordmann, STEC(http://shigatox.net/new/reference-strains/ecor.html)	100mls		Bleach	Y
Escherichia coli (Shiga-toxin producing and pathogenic)	DECA collection, EHEC 933W, EPEC, ATCC 700927, ATCC 43888, ATCC BAA-2196, ATCC BAA-2193, ATCC BAA-2193, ATCC BAA-2215, ATCC BAA-2440, ATCC BAA-2219, ATCC BAA-2192	2	ATCC, SGSC, STEC (http://shigatox.net/new/reference-strains/deca.html)	100mls		Bleach	Y
Escherichia coli B	BL21, BL21(DE3), Rosetta, Rosetta(DE3), BL21AI, BB, REL606, BL21(DE3)/pLysS, SHuffle express T7, Express Iq	1	ATCC, NEB, Lucigen, Invitrogen, TKL	100mls		Bleach	N
Escherichia coli C	C-1, C-1792 , C-236 , C-367 , C-368	1	Ian Molineux, ATCC, TKL	100mls		Bleach	N

Escherichia coli	rcF471-6, rcF479, rcF513-9	1	Mouse fecal isolates	100mls		Bleach	N
Klebsiella oxytoca	**CCUG 15788**	1	Sherwood Casjens	100mls		Bleach	N
Klebsiella pneumoniae	sp. 390, MGH78578, K6 / ATCC 700603 (SHV-18), CDC1000527 (NDM-1), 1100975 (NDM-1), 1002565 (NDM-1), 1100770 (NDM-1), KPNIH1, 10, 24, 27, 29, 30, 31, 32, 33, Kp80	2	ATCC, TKL, Julie Segre, Ian Molineux, Eliava Institute	100mls		Bleach	Y
Pseudomonas aeruginosa	PAO1, PAK, PA14, ATCC 10145, CECT111, ATCC25102, rcF477, rcF478, Ps25, Ps32	2	ATCC, Ribbeck lab, mouse fecal isolates, Eliava Institute	100mls		Bleach	Y
Pseudomonas aurantiaca	planned	1	ATCC	100mls		Bleach	N
Pseudomonas chlororaphis	planned	1	ATCC	100mls		Bleach	N
Pseudomonas citronellolis	planned	1	ATCC	100mls		Bleach	N
Pseudomonas fluorescens	A506	1	ATCC, TKL	100mls		Bleach	N
Pseudomonas putida	*KT2440, C1S, ATCC12633*	2	ATCC, TKL	100mls		Bleach	Y
Pseudomonas veronii	planned	1	ATCC	100mls		Bleach	N
Pseudomonas cellulosa	ATCC55703	1	ATCC	100mls		Bleach	N
Enterococcus faecium	JCM5804	2	R. Rahmeh	100mls		Bleach	Y
Salmonella enterica enterica Agona	SARB1	2	Salmonella enterica enterica Genetic Stock Center, ATCC, TKL	100mls		Bleach	Y
Salmonella enterica enterica Anatum	SARB2, ss44, 15/5	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica Bongori	SP141, SP905	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Brandenburg	SARB3	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Choleraesuis	SARB6, SARB7, SC-B67, 14174, A50, G9	2	SGSC, ATCC, John Elmerdahl olsen	100mls		Bleach	Y
Salmonella enterica enterica decatur	SARB8	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Derby	SARB9, SARB10	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Dublin	SARB13, SARB14, TY3627, SGSC4916,	2	SGSC, ATCC, John Olsen	100mls		Bleach	Y

	SGSC4157, 3246						
Salmonella enterica enterica Duisberg	SARB15	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Emek	SARB20	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica enteritidis	SARB18, SARB19, LK5, ATCC 13076	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Enteritidis	Kuwait (MDR poultry isolate)	2	R. Rahmeh	100mls		Bleach	Y
Salmonella enterica enterica Gallinarum	SARB21, 287/91	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Hadar	SL485	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Heidelberg	SARA30, SARA36 to SARA40, SARB24, SL486	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Indiana	SARB25	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Kentucky	#98, SP146	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Miami	SARB26, SARB29	2	SGSC, Ian Molinzux, John Elmerdahl Olsen	100mls		Bleach	Y
Salmonella enterica enterica Montevideo	SARB31	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica münchen	SARA63 to SARA67, SARA69, SARA70, SARA72, SARB33	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Newport	SARB37	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Panama	SARB51, SARB52	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Reading	SARB53	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Rubislaw	SARB54	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Saint-Paul	SARA24, SARB55, SARB56	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Schwarzengrund	CVM19633	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Sendai	SARB58	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Senftenberg	SARB59	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Thompson	SARB62	2	SGSC, ATCC	100mls		Bleach	Y
Salmonella enterica enterica Typhimurium	LT2, IJ612, MK1046, LT1 to LT6, LT7 to LT9, A36, DT4a, LT11, LT13, LT14, LT16 to LT21, SARA1 to SARA10,	2	SGSC, ATCC, John Elmerdahl olsen, Lionello Bossi, Sébastien Lemire	100mls		Bleach	Y

	SARA12, SARA24, SARB66 to SARB68, 4/74, CVM23701, M8c, TT23381, C5, SP296, SP358, SP394, SP513, SP591, SP592, SP594, SP651, SP71, SP784, SP811, SP838, SP839, SP906						
Salmonella enterica enterica Typhisuis	SARB69	2	SGSC, ATCC	100mls		Bleach	Y
Serratia marcescens	planned	2	ATCC	100mls		Bleach	Y
Serratia plymuthica	V4	1	ATCC, TKL	100mls		Bleach	N
Shigella boydii	ATCC 9207	2	SGSC	100mls		Bleach	Y
Shigella dysenteriae	planned	2	ATCC, Fasano lab (MGH)	100mls		Bleach	Y
Shigella flexneri	ATCC 12022, M16, 2457T	2	SGSC, ATCC	100mls		Bleach	Y
Shigella sonnei	ATCC 25931, ATCC 9290	2	SGSC, ATCC	100mls		Bleach	Y
Staphylococcus aureus	RN4220	2	L. Marraffini	100mls		Bleach	Y
Stenotrophomonas maltophilia	planned	2	ATCC	100mls		Bleach	Y
Streptococcus agalactiae	planned	2	ATCC	100mls		Bleach	Y
Streptococcus mutans	planned	1	ATCC	100mls		Bleach	N
Streptomyces coelicolor	DSMZ 41109, DSMZ 1042, DSMZ 41546	1	Kolter lab, DMSZ	100mls		Bleach	N
Yersinia pseudotuberculosis	YPIII, IP2666	2	ATCC, Joan Mecsas	100mls		Bleach	Y
Yersinia enterocolityca	planned	2	ATCC	100mls		Bleach	Y

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

Organism	**Infectious Dose**	**Route of exposure**	**Host range**	**Clinically relevant Drug resistance**	**Availability of treatment**	**Special equipment /PPE used (above standard PPE)**	**Disease state and symptoms of exposure**
Bacteroides thetaiotaomicron; B. ovatus; B. uniformis; Parabacterioides distastonis; Bacteroides caccae; B. eggerthii;	Unknown	Mucosal contact; injection/ non-intact skin	Humans, dogs, cats, and other animals		Metronidazole, imipenem, and amoxicillin seem to be effective against B. fragitis and B. thetaiotaomicron [16]. Studies using a rat model have shown that pretreatment using oral	N/A	Bacteroides spp. represent an important anaerobic bacterial genus associated with human infections[3]. In combination with other facultative/strict anaerobes, they are responsible for the majority of localized

					vancomycin/imipenem resulted in undetectable levels of Bacteroides spp		abscesses within the cranium, thorax, peritoneum, liver, and female genital tract[4,8]. They can cause pulmonary abscesses when naturally-occurring oropharangeal Bacteroides and closely related genera are aspirated into the lung[8]. These taxa can lead to many types of diseases, some of which can be fatal, including noma (cancrum oris), human apical periodontitis, endocarditis, pelvic inflammatory disease, suppurative thrombophelebitis, and wound infections[4,6,9]. Organisms from oral flora also have a role in dental abscesses and infectivity of human bites. Bacteroides fragilis is the most common opportunistic pathogen of Bacteroides spp.[1,4]. Spread to bloodstream (bacteremia) is more common for B. fragilis than any other anaerobe[4]. Deep pain and tenderness below the diaphragm are typical of B. fragilis infection. Widespread intra-abdominal abscesses may be associated with fever and abdominal pain.
Pseudomonas (aeruginosa, putida, fluorescens)	Unknown	Ingestion, Injection/ non-intact skin	Humans	Species resistant to many antibiotics	Species susceptible to extended-spectrum penicillins (such as ticarcillin, azlocillin, and piperacillin), aminoglycosides, cephalosporins, fluoroquinolones, polymixins, and the monobactams	N/A	As opportunistic pathogens, *Pseudomonas* spp. often invades the host tissue and cause infection and bacteremia in immunocompromised hosts (e.g., HIV/AIDS, cystic fibrosis, bronchiectasis, and severe chronic obstructive pulmonary disease, burns, malignancy, or diabetes mellitus). The common site of infection is the lower

							respiratory tract, and severity ranges from colonization without immunological response to severe necrotizing bronchopneumonia; such severe infection in patients with cystic fibrosis is almost impossible to eradicate once established in the airways. Pseudomonal pneumonia often develops from oro-pharyngeal contamination or secondary bacteremia, and is also a common cause of nosocomial ventilator-related pneumonia in intensive care settings. Infections also include endocarditis, osteomyelitis, urinary tract infections, gastrointestinal infections, meningitis, and, commonly, septicaemia. *P. aeruginosa* is the most common agent associated with infection and inflammation during contact lens wear. The bacteria colonize on lenses and produce proteases to kill or invade corneal cells, an infection that can lead to scarring and vision loss. The species is also the most virulent with a mortality rate of 30%, which can be higher depending on predisposing conditions. *P. aeruginosa* can also readily colonize on open burn wounds, causing infections, abscesses, and sepsis, with edema and/or discoloration of unburned skin at wound margins and green pigment in subcutaneous fat. *P. aeruginosa* is also associated with swimmer's ear (otitis externa). Other *Pseudomonas* sp

							ecies are also opportunistic; however, cases of infection are rare.
Clostridium symbiosum, C. spiroforme		Injection/ non-intact skin	Humans		most species are susceptible to penicillin, clindamycin, chloramphenicol, piperacillin, metronidazole, imipenem, and combinations of b-lactams with b-lactamase inhibitors.		Illnesses primarily associated with Clostridium spp. are: Clostridial bacteremia: Symptoms can vary greatly but will typically include fever, chills, and leukocytosis. The fatality rate ranges from 25-50%. Many Clostridium spp. can be associated with anaerobic bacteremia including C. septicum, C. ramosum, C. clostridioforme, or C. tertium
ENTEROCOCCUS FAECALIS	Unknown	Ingestion; mucosal contact; injection/ non-intact skin	Humans	Strains resistant to β-lactams, aminoglycosides and, increasingly, vancomycin have been described[2,4]. Strains have also been identified which carry genetic elements conferring resistance to chloramphenicol, tetracyclines, macrolides, lincosamides, quinolones, and streptogramins[2].	Most strains remain susceptible to penicillin, ampicillin, and vancomycin.		Enterococci can cause urinary tract, wound, and soft tissue infections[2,4]. They are also associated with bacteremia which can lead to endocarditis in previously damaged cardiac valves[4]. E. faecalis is the most frequent species isolated from human intestine samples (80-90%), E. faecium accounts for 5-10% of isolates[1
Salmonella enterica (non typhoidal)	For non-typhoidal salmonellosis, the infectious dose is approximately 10^3 bacilli [4, 7]. For enteric fever, the infectious dose is about 10^5 bacilli by ingestion [4, 6, 7]. Patients with achlorhydria, depressed cell-mediated immunity, or who are elderly may become infected with at a lower infectious dose [4, 7]. The infectious	Ingestion; Injection/ non-intact skin	Humans	Some resistance to chloramphenicol has been reported and, in 1989, 32% of strains were multi-drug resistant	Susceptible to chloramphenicol, ciproflaxin, amoxicillin, co-trimoxazole, trimethprim-sulfonamid, cephalosporins and norfloxacin		Salmonella enterica can cause four different clinical manifestations: gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state [7]

	dose may also be dependent on the level of acidity in the patient's stomach						
Stenotrophomonas maltophilia	Unknown	Ingestion; mucosal contact; injection/ non-intact skin	Humans	Species is not usually susceptible to piperacillin, and susceptibility to ceftazidime is variable	Many strains of S. maltophilia are sensitive to co-trimoxazole and ticarcillin,	N/A	S maltophilia has few pathogenic mechanisms and, for this reason, predominantly results in colonization rather than infection. If infection does occur, invasive medical devices are usually the vehicles through which the organism bypasses normal host defenses. Otherwise, the pathophysiology of this nonfermentative aerobic gram-negative bacillus does not differ from other nonfermentative aerobic organisms
Streptococcus agalactiae	Unknown	Mucosal contact; Injection/ non-intact skin	Humans	some strains penicillin tolerant and require treatment with an aminoglycoside as well.	Sensitive to penicillin or ampicillin		It presents with nonspecific symptoms, such as fever, vomiting and irritability, and can consequently lead to late diagnosis. Hearing loss can be a long-term sequela of group B Streptococcus species (GBS)-meningitis. Infection with GBS is the cause of some instances of stillbirth.
Klebsiella pneumoniae	Unknown; according to one source may be 10^8 Klebsiella organisms per gram of feces are required to produce damage	Ingestion; mucosal contact; inhalation ; injection/ non-intact skin	Humans	Since more and more strains of Klebsiella spp. appear to be developing and harbouring extended-spectrum beta-lactamases (ESBLs), cephalosporinases, and carbapenemases, resistance of Klebsiella spp. to current antibiotics appears to be increasing these strains are not pan-resistant to all beta-lactam antibiotics, but have differing susceptibility protocols, so that in some cases beta-	Klebsiella spp. are known to show resistance to penicillins, especially ampicillin and carbenicillin Other treatment regimes include beta-lactams in combination with a beta-lactamase inhibitor, tigecycline, aztreonam, and colistin. According to results from some studies in Europe and USA, ranges of susceptibility were as follows [1]: ceftazidime (92-95%), ceftriaxone (96-98%),		Respiratory Disease: K. pneumoniae – a leading cause of community-acquired and nosocomial pneumonia and lung abscesses. Infection of the upper lobe is more common. Symptoms include: fevers, chills, and leukocytosis with red currant jelly-like sputum [1]. Rare complications include lung infection involving necrosis and sloughing of the entire lobe. Central nervous system (CNS) infections: K. pneumoniae – cause community-acquired meningitis and brain abscesses. Clinical symptoms include: headaches, fever, altered conciousness,

				lactams can still be used to treat them, although in high concentration. Other treatment regimes include beta-lactams in combination with a beta-lacatmase inhibitor, tigecycline, colistin and aztreonam	cefotaxime (96%), piperacillin-tazobactam (90-97%), imipeneum (98-100%), gentamicin (95-96%), amikacin (98-99%), triethoprimsulfamethoxazole (SXT) (88-90%).		seizures, and septic shock. Hepatic disease: K. pneumoniae – an important causative pathogen for pyogenic liver abscesses with symptoms including fever, right-upper-quadrant pain, nausea, vomiting, diarrhoea or abdominal pain, and leukocytosis. Abscesses occur predominantly in the right lobe and are solitary
Shigella flexneri, dysenteriae and boydii	10-200 organisms	Ingestion; mucosal contact; inhalation ; injections /non-intact skin	Humans	Multidrug-resistant strains are emerging, including those against trimethoprim-sulfamethoxazole (TMP-SMX), ampicillin, and chloramphenicol	Susceptible to ampicillin, trimethoprim, sulfamethoxazole, naldixic acid, ofloxacin, chloramphenicol, fluoroquinolones, and ciprofloxacin		Infection may be mild and asymptomatic, but it is most commonly characterized by acute intestinal infections upon ingestion, resulting in mild watery diarrhea to severe inflammatory bacillary dysentery or shigellosis, manifested by severe abdominal cramps, nausea and vomiting, fever, tenesmus, anorexia, and stool containing blood and mucus (1, 2, 8). Further complications include Reiter's syndrome which has been associated with S. flexneri (9, 10), severe dehydration, intestinal perforation, toxic mega colon, bacteremia, toxaemia (11), septicaemia, seizures, toxic encephalopathy with headache and alterations of consciousness, septic shock and convulsions (very rare) (4), and haemolytic uremic syndrome, which have been linked to Shiga toxin (a potent cytotoxin produced by S. dysenteriae that can also cause other neurotoxic effects). Virulence of Shigella is temperature-regulated, as organisms are able to invade HeLa cells at 37°C, and cannot do so in vitro at 30°C (12).

							Infections are usually self-limiting, but can become life-threatening in immunocompromised patients or if not properly treated. Severity of infection depends on the host, dose, and serotype [(2)]. S. dysenteriae is the most pathogenic species, with a fatality rate up to 20%, whereas S. sonnei usually cause mild forms of shigellosis.
E. coli other than laboratory strains, EHEC, STEC, EPEC, the ECOR collection	Ranges from 10-100 for EHEC strains to 10^6-10^8 for other strains	Ingestion; mucosal contact; inhalation ; injection/ non-intact skin	Humans; cattle, all animals	Some of these strains are carbapenem-resistant, but not pan-resistant to antibiotics.	Therapeutic treatment E coli meningitis requires antibiotics, such as third-generation cephalosporins (eg, ceftriaxone). E coli pneumonia requires respiratory support, adequate oxygenation, and antibiotics, such as third-generation cephalosporins or fluoroquinolones. E coli cholecystitis/cholangitis requires antibiotics such as third-generation cephalosporins that cover E coli and Klebsiella organisms. Empiric coverage should also include anti– E faecalis coverage. For E coli intra-abdominal abscess, antibiotics also must include anaerobic coverage (eg, ampicillin and sulbactam or cefoxitin). In severe infection, piperacillin and tazobactam, imipenem and cilastatin, or meropenem may be used. Combination therapy with antibiotics that cover E coli plus an antianaerobe can also be used (eg, levofloxacin plus		Beneficial strains of E. coli typically colonize the infant gastrointestinal tract within a few hours after birth. The presence of this bacterial population in the intestine suppresses the growth of harmful bacteria and is important for synthesizing appreciable amounts of B vitamins. E. coli usually remains harmless when confined to the intestinal lumen. However, in debilitated or immuno-suppressed humans, or when gastrointestinal barriers are violated, even normal, "non-pathogenic" strains of E. coli can cause infection. It is also known that some E. coli strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system in even very healthy people. Strains of E. coli that cause diarrhea include strains that cause traveler's diarrhea (enterotoxigenic E. coli), persistent diarrhea (enteroaggregative E. coli), watery diarrhea of infants (enteropathogenic E. coli), hemorrhagic colitis (bloody

					clindamycin or metronidazole). E coli enteric infections require fluid replacement with solutions containing appropriate electrolytes. Antimicrobials known to be useful in cases of traveler's diarrhea include doxycycline, trimethoprim/sulfa methoxazole (TMP/SMZ), fluoroquinolones, and rifaximin. They shorten the duration of diarrhea by 24-36 h. Antibiotics are not useful in enterohemorrhagic E coli (EHEC) infection and may predispose to development of HUS. Antimotility agents are contraindicated in children and in persons with enteroinvasive E coli (EIEC) infection. Uncomplicated E coli cystitis can be treated with a single dose of antibiotic or 3-d course of a fluoroquinolone, TMP/SMZ, or nitrofurantoin. Recurrent E coli cystitis (ie, >2 episodes/y) is treated with continuous or postcoital prophylaxis with a fluoroquinolone, TMP/SMZ, or nitrofurantoin. Patients with complex cases (eg, those with diabetes, >65 y, or recent history of UTI) are treated with a 7- to 14-d course of antibiotics (eg, levofloxacin, third-generation cephalosporins, or aztreonam).		diarrhea), and hemolytic uremic syndrome (enterohemorrhagic E. coli) Shiga toxin-producing E. coli (STEC) can cause the following symptoms: Nausea Severe abdominal cramps Watery or very bloody diarrhea Fatigue STEC can also cause low-grade fever or vomiting. Symptoms usually begin from 2 to 5 days after eating contaminated food or drinking contaminated liquids. Symptoms may last for 8 days, and most people recover completely from the disease.

					Acute uncomplicated E coli pyelonephritis in young women is treated with fluoroquinolone or TMP/SMZ for 14 d. Patients with vomiting, nausea, or underlying illness (eg, diabetes) should be admitted to the hospital. If fever and flank pain persist for more than 72 h, ultrasonography or CT scanning may be performed. Treat E coli perinephric abscess or prostatitis with at least 6 wk of antibiotics. E coli sepsis requires at least 2 wk of antibiotics and identification of the source of bacteremia based on imaging study results. McGannon et al found that antibiotics that target DNA synthesis, such as ciprofloxacin (CIP) and TMP/SMZ, showed increased Shiga toxin production, whereas antibiotics that target the cell wall, transcription, or translation did not. Remarkably, high levels of Shiga toxin were detected even when growth of O157:H7 was completely suppressed by CIP. In contrast, azithromycin significantly reduced Shiga toxin levels even when O157:H7 viability remained high. Extended-spectrum cephalosporins are widely administered to treat serious infections due to gram-negative bacilli. However,		

					managing infections due to ESBL-producing isolates is particularly challenging since these isolates have varying levels of resistance to agents in the extended-spectrum cephalosporin class, in addition to being multiply resistant to other antimicrobials such as aminoglycosides, sulfonamides, and fluoroquinolones A MEDLINE search was performed for all studies published in the English literature using key words "ESBL" and "extended-spectrum ß-lactamase." Studies that provided treatment information on patients infected with a putative or known ESBL-producer were evaluated. An isolate tested positive with the double-disk synergy test and/or Etest strips (AB Biodisk) was considered a putative ESBL producer unless molecular analysis was performed to characterize the enzyme type(s).		
YERSINIA PSEUDOTUBERCULOSIS	10^8 bacteria or more orally	Ingestion; injection/ non-intact skin	Humans; rabbits, rodents, cattle, pigs, pets, wild mammal and birds	Unknown	Susceptible to ampicillin, third generation cephalosporins, aminoglycosides, tetracyclines, and chlorampheinicol.		Yersinia pseudotuberculosis is a rare cause of acute enteric disease with symptoms such as acute mesenteric lymphadenitis and gastroenteritis associated with abdominal pain and fever (diarrhea is unusual). One to 3 weeks after the acute phase of the disease, post-infectious complications can occur, such as reactive arthritis and erythema nodosum. The arthritic

							phase of the disease can last up to 6 months. Other complications include lesions to lymph nodes, spleen and liver, as well as septicaemia in immunocompromised patients. The disease is most common in children and young adults and immunocompromised individuals are at greater risk of severe disease or death.
YERSINIA ENTEROCOLITICA	10^8 bacteria or more orally	Ingestion; injection/ non-intact skin	Humans; farm animals, birds, pets	It is generally resistant to penicillin and its derivatives and to narrow spectrum cephalosporins	Yersinia enterocolitica is susceptible to chloramphenicol, fluoroquinolones, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole.		Yersinia enterocolitica infection is characterized by enteritis, enterocolitis (particularly in children), fever (39ºC), watery stools, abdominal pain and acute mesenteric lymphadenitis (which may mimic appendicitis). In some cases acute terminal ileitis and enteric fever can occur. 1-3 weeks after the initial clinical symptoms, reactive arthritis and erythema nodosum may occur which can last about 6 months after infection. In rare instances, complications can include meningitis, endophthalmitis, conjunctivitis, myocarditis, pneumonia, pulmonary abscess, hepatitis, cholangitis, peritonitis, glomerulonephritis, urethritis, cellulitis, haemolytic anaemia, thyroiditis, pharyngitis and septicaemia
Clostridium difficile	Unknown	Ingestion	Humans, pigs, calves	Some rare strains are resistant to metronidazole; Fluroquinolone-resistant hypervirulent strain 027 isolates in US	Susceptible to metronidazole, oral vancomycins; penicllins and cephalosporins in vitro		C. difficile is the main cause of nosocomial antibiotic- associated diarrhea. Antibiotics or any other procedures that disrupt the normal intestinal microbiota can lead to the overgrowth and production of toxin by C. difficile, which leads to clinical manifestations of infection. The diarrhea

							may range from a few days of intestinal fluid loss to life-threatening pseudomembranous colitis. In diarrhea without pseudomembranous colitis (PMC), feces have a foul odor and are not bloody. Abdominal pain with or without pyrexia may also be present along with diarrhea. PMC is associated with intense inflammation of the colon and formation of pseudomembranes on the intestinal mucosal surface. Patients with PMC also have more systemic side effects.
ENTEROBACTER AEROGENES and cloacae	Unknown	Ingestion; injection/ non-intact skin	Humans	Enterobacter spp. are resistant to ampicillin; first- and second- generation cephalosporins[7]; and cephalothin(	Most Enterobacter spp. are susceptible to cefepime[7], aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole[8]. Tigecycline has been shown effective in vitro		Enterobacter spp., particularly E. aerogenes and E. cloacae, have been associated with nosocomial outbreaks, and are considered opportunistic pathogens[1,5]. Enterobacter spp. can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections[6,7]. In addition, Enterobacter spp. have been noted in intravascular device-related infections, and surgical site infections (primarily postoperative or related to devices such as biliary stents)[7]. Many species can cause extra-intestinal infections[6], for example, Enterobacter sakazakii, has been associated with brain abscesses in infants and with meningitis[3,7]. Mortality rates for bacterial meningitis range from 40-80%[5].

SERRATIA MARCESCENS and PLYMUTHICA	Unknown	Injection/ non-intact skin	Humans	Many Serratia spp. isolates (39-73%) are resistant to gentamicin. They are all resistant to penicillins and cephalosporin	Serratia spp. are usually susceptible to aminoglycosides, fluoroquinolones, and co-trimazole		Serratia spp. are opportunistic pathogens and are one of the ten most common causes of bacteremia in North America. They are responsible for a variety of infections, including bacteremia, pneumonia, intravenous catheter-associated infections, osteomyelitis, endocarditis, and, rarely, endogenous and exogenous endophthalmitis. Symptom of endophthalmitis appears rapidly after infection, and may include fever, erythema, ocular pain, periorbital swelling, and hypopyon (pus in the eyes). The mortality rate from bacteremia due to Serratia spp. 6 months after infection is 37%.

a2. List and describe any other biological material not covered in the tables above:

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

1. Briefly describe experiment and procedures involving use of biological agents?

All bacteria will be grown on standard media, using conventional protocols as described in Current Protocols in Molecular Biology[2.]. As described above, selected species will be transformed using electroporation. E coli lab strains may be transformed using chemical competence.

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:

As described above, bacteria will be modified to produce novel compounds or to produce higher amounts of normal metabolites. Specified bacteria will receive antibiotic resistance for selection, but no pathogen will be modified to become resistant to a clinically relevant antibiotic.

3. Will you introduce viable biological material (microorganisms or cells) into animals?

   Yes ☒ No ☐ If so:

   a) Please describe:

0915-092-18:
We will introduce recombinant organisms, including engineered E. coli, B. thetaiotaomicron, B. fragilis, B. ovatus and B. vulgatus strains, as well as the probiotic yeast S. boulardii into the mouse gastrointestinal tract. The objective is to test genetically engineered strains for their ability to detect gut inflammation and/or secrete anti-inflammatory proteins, for their ability to act as as next-generation of inflammation bowel disease sensors and therapeutics. Organisms will be introduced by oral gavage. Recipient species includes Balb/c mice, SJL mice, and C57BL/6 mice. No engineered bacterial strains are known to have or will acquire virulence. The bacteria may have display resistance to common laboratory antibiotics such as kanamycin, or erythromycin, but not to clinically relevant drugs.

1215-114-18:
Development of Therapeutic Bacteriophage for Decolonization: A major concern in infectious disease is the establishment of antibiotic resistance and pathogen reservoirs in healthy individuals that could potentially later cause life threatening opportunistic infections. We wish to explore the use of bacteriophage as a means to specifically eliminate antibiotic resistant or pathogenic strains from a microbiome without affecting commensal populations. We intend first demonstrate this concept using non-pathogenic *E. coli* strains (K-12, BL21, C-1) and *Klebsiella spp.* (sp. 390 and *oxytoca*). Later, we extend this concept to clinical isolates carbapenem-resistant Enterobacteriaceae (CREs), including *E. coli* and *K. pneumoniae* strains. As many of these human isolates do not naturally colonize the mouse gut, we will perturb the microbiota using antibiotic treatment (streptomycin or ampicillin) to allow our strains to colonize.

1016-064-19

The goal is determine whether different agents such as ionic liquids, nitroxides and antimicrobial peptides exhibit anti-infective properties *in vivo* in two skin infection models, superficial and abscess. There is a need for alternative therapeutics to treat drug- resistant infections. Here, we aim to test the efficacy of novel classes of antimicrobials in mouse models

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: _____ **0915-092-18; 1215-114-18, 1016-064-19.**

Section 3. Research Description (required): Project 3 – Insertion of Synthetic Circuits into E. coli

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

Synthetic Biology is premised on the idea that an organism can be thought of as a living computer. Inputs are received and relayed through logic circuits to produce desired outputs. Here we describe a number of projects designed to develop tools for engineering the model organism E coli, or for using those tools to perform computational processes.

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.

3.1) Identification of safe insertion sites for synthetic circuits in the genome of *E. coli* and optimization of gene expression in *E. coli* using MAGE (Multiplex Automated Genomic Engineering) technology.

The recombination functions of phage lambda, known as lambda red show the unusual capacity to catalyze the recombination between short linear DNA segments allowing the introduction of relatively long double stranded linear DNA constructs (>5kb) or short single stranded DNA (>200bp) fragments with high efficiency and precision as long as they display short regions of homology to the target locus (25-50bp) at each end. This technique, known as recombineering, has revolutionized genomic engineering in *E. coli* and many other related bacteria but very little is known about the factors that affect precision or efficiency of the recombination event leading to DNA insertion.

With this project, we are studying 1) whether there is an efficiency bias from targeted locus to targeted locus 2) identifying safe insertion sites where the transgene being inserted is either more active or less perturbed by the surrounding genetic context.

1) We are generating a library of small construct composed of a resistance marker (kanamycin) flanked by 50bp homology regions directing them to ~150 different genetic loci spread throughout the genome. After transformation of this bank into recombineering proficient *E. coli k-12*, we are recovering the population and analyzing the frequency of insertion at each independent locus by high throughput sequencing and compare this to the expected insertion frequency calculated from the abundance of each different construct in the input DNA. If a bias is discovered at some positions, we will attempt to correlate it with characteristics of the DNA surrounding these particular insertion sites.

2) A small construct composed of a resistance marker (kanamycin, spectinomycin or chloramphenicol) and a reporter gene with an inducible promoter is targeted to various loci throughout the genome of *E. coli* k-12 by recombineering. The resulting strains are then compared to evaluate the level of expression of the reporter (GFP) with and without inducer in order to identify regions where insertion of a transgene is least affected by surrounding genes while allowing high-level expression.

3.2) High efficiency targeted genome evolution

We are developing a system for high-efficiency genome editing in bacteria via in vivo expression of ssDNA by reverse transcription from a template plasmid followed by recombineering (SCRIBE). This is similar to the previously published recombineering techniques (e.g. MAGE) that are widely used for genome engineering in bacteria, but instead of delivering oligos from outside, we produce the ssDNA inside the cells using a bacterial class of reverse transcriptases called retrons. This allows us to specifically introduce desired small (i.e. a few bps) modifications into selected genomic loci. As a proof of concept, we are performing experiments in *E. coli* K-12 (DH5alpha and MG1655). We are expressing the retron cassette and beta recombinase and introducing modifications into endogenous loci of *E. coli* K-12 (*galK* and *lacZ*) as well as an integrated *aph* marker, which confers resistance to kanamycin. We use CRISPRi technology to knockdown *E. coli* endogenous exonucleases to increase intracellular stability of the expressed ssDNA and increase the recombination efficiency. We also use CRISPR nuclease to counter-select against the WT allele and thus increase the efficiency of editing. We use this gene editing platform to demonstrate continuous evolution of a trait of interest (ability to metabolize lactose) by continuously introducing new mutations into *lac* locus of *E. coli* MG1655 and selecting for the cells that can grow faster in minimal media + lactose by serial passage of cells in minimal media + lactose.

The experimental procedures will include introduction of recombinant DNA encoded on plasmid vectors, via different delivery methods including transformation, conjugation and transduction (phagemid delivery) into *E. coli* genome as well as modification of *E. coli* genome, necessitating the use of antibiotic markers. The antibiotic markers will confer resistance to chloramphenicol, kanamycin, spectinomycin, streptomycin, or ampicillin. The model organism *E. coli* MG1655 will be used as proof of concept. This strain is a commonly used BL1 laboratory organism, and poses no threat to human health. We also plan to demonstrate the generalizability of the genome editing approach by investigating the genome editing approach In Pseudomonas putida KT2440. We will express the beta recombinase and retron cassette in this organism and use the *upp* gene as a selectable marker to measure the efficiency of our system in this organism.

3.3) DNA as a storage medium: cryptographic approaches to data protection.

Synthetic DNA has great propensity for efficiently and stably storing non-biological information. With DNA writing and reading technologies rapidly advancing, new applications for synthetic DNA are emerging in data storage and communication. Traditionally, DNA communication has focused on the encoding and transfer of complete sets of information. Here, we explore the use of DNA for the communication of short messages that are fragmented across multiple distinct DNA molecules. We identified three pivotal points in a communication-data encoding, data transfer & data extraction-and developed novel tools to enable communication via molecules of DNA. To address data encoding, we designed DNA-based individualized keyboards (iKeys) to convert plaintext into DNA, while reducing the occurrence of DNA homopolymers to improve synthesis and sequencing processes. To address data transfer, we implemented a secret-sharing system-Multiplexed Sequence Encoding (MuSE)-that conceals messages between multiple distinct DNA molecules, requiring a combination key to reveal messages.

To address data extraction, we achieved the first instance of chromatogram patterning through multiplexed sequencing, thereby enabling a new method for data extraction. We envision these approaches will enable more widespread communication of information via DNA.

3.4) **Crosstalk correction in gene circuits in *E. coli***:

This work aims to develop an easy and generalizable way of correcting crosstalk that occurs in multi input/output synthetic gene networks within *E. coli*. As proof of concept, we will express several transcription factors as sensors in *E. coli*, namely OxyR (sensing H_2O_2), SoxR (sensing superoxide or paraquat), LuxR (sensing C6-HSL), LasR (sensing C12-HSL) and RhlR (sensing C4-HSL). The reporter construct consists of a promoter for the transcription factor (pOxyR, pSoxS, pLux, pLas, pRhl) and mCherry or sfGFP as a reporter gene. Plasmids carry an origin of replication for *E. coli* (pSC101, p15a or ColE1) and a resistance cassette (Kanamycin, Carbenicillin/Ampicillin, Spectinomycin, Chloramphenicol). This work is only done *in vitro* and strains will not be tested in mice.

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. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OSP website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

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 (organism the sequence is from), promoter used, nature of insert (genes families are acceptable, e.g. fluorescent proteins), if 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
Synthetic DNA	Vectors from the Lutz and Bujard collection[1.]	N	Rational design	Short homology to E coli genome	N	0
Synthetic DNA	Vectors from the Lutz and Bujard collection[1.]	N	Rational design	Secret code	N	0
Resistance genes such as kanR	Vectors from the Lutz and Bujard collection[1.]	constitutive	Plasmid library	Antibiotic resistance	Y	0
Reporter such as GFP	Vectors from the Lutz and	inducible	Plasmid library	Fluorescent protein	Y	0

	Bujard collection[1.]					
Sensors including OxyR (sensing H_2O_2), SoxR (sensing superoxide or paraquat), LuxR (sensing C6-HSL), LasR (sensing C12-HSL) and RhlR (sensing C4-HSL)	Vectors from the Lutz and Bujard collection[1.]	constitive	E coli	Transcription factors	Y	0
Reporters such as mCherry	Vectors from the Lutz and Bujard collection[1.]	pOxyR, pSoxS, pLux, pLas, pRhl	Plasmid library	Fluorescent proteins	Y	0

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

3. Do any sequences code for toxins not covered in (2) above? 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 used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, 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 strain	Reporter gene?	% viral genes	Type of promoters	Host cell
Vectors from the Lutz and Bujard collection[1.]	E coli K12, MG1655	Sometimes fluorescent protein, as described above	0	Constitutive, inducible such as pOxyR, pSoxS, pLux, pLas, pRhl (described in detail above	E coli K12 or MG1655

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: https://cabescro.mit.edu/home

Viral Vector type	
Description (Plasmids used, viral vector generation for lentiviral vector)	
Source (vendor / collaborator)	
Packaging cell line(s), if applicable	
Replication competent or incompetent	
Assays for detecting replication competent virus, if applicable	
Pseudotype	
Host range	
Safety feature (e.g. self-inactivating)	
Integrate into genome (yes/no)	
Exposure hazard (e.g. insertional mutagenesis)	
Promoters to be used with viral vector	
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

3. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

a. 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 to register your animal research; If you have CAC protocol no(s), list them here:______________________. 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? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

5. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

6. Describe the steps used to handle and contain spills:

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/animals/plants/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 used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Escherichia coli K-12	MG1655, DH5alpha, EMG2, KL463, XL10, C600, DH10B, BW25113, DH5alpha Pro, MG1655 Pro, RFS289, CJ236, ER2738, ER2267, NEB10beta, E. cloni 10G, XL1-GOLD, XL1BLUE, XL1-RED, W3110, NM2, S17, CR63, CR63l, ME5486, transformax EC100D pir, Transformax EC100D pir-116, SURE2, MK01, MK02, ET12567,	1	CGSC, NEB, ATCC, Thermo, Lucigen, Keith Shearwin, Laub lab, Collins lab, Agilent, expressys, invitrogen, epibio, clontech, Roberto Kolter, real biotech corporation, the Yale E. coli genetic stock center	100mls		Bleach	N

	C600, W1, MC4100, oneshot TOP10, SHuffle-T7, MG1655(DE3), NEB10beta, MG1655 ΔntrBC, MG1655Pro with pLtetO-mf-Lon protease integrated, Transformax EPI3000, DH5alpha F' iq, sbtl3, sbtl4, stellar, HIT DH5-alpha, JM109						

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

Organism	Infectious Dose	Route of exposure	Host range	Clinically relevant Drug resistance	Availability of treatment	Special equipment /PPE used (above standard PPE)	Disease state and symptoms of exposure

a2. List and describe any other biological material not covered in the tables above:

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

4. Briefly describe experiment and procedures involving use of biological agents?

Bacteria are grown and transformed using standard protocols, as described in Current Protocols in Molecular Biology[2].

5. 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:

Antibiotic resistance

6. Will you introduce viable biological material (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 3. Research Description (required): Project 4 Synthetic Biology of Mycoplasma genitalium

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

Mycoplasma is a very simple bacterium with a small genome. As such it has the potential to be a useful model organism. However, there is a lack of basic tools to use to engineer Mycoplasma. We are endeavouring to develop these tools.

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.

The goal of this work is to improve our ability to rationally engineer biological systems which requires more comprehensive models for prediction of phenotypes based on genomics, proteomics and metabolomics datasets. The whole-cell model of *mycoplasma genitalium* is the most complete model of an organism to date. We aim to investigate the use of this model to see how such models can allow us to better engineer organisms.

The work envisions working with the human pathogen *Mycoplasma Genitalium* (primarily strain G37, ATCC designation 33530, but also additional wild-type strains if necessary), which is a BL2 organism.

Mycoplasma are both important pathogens and useful model organisms. Derivatives of MiniTn4001PsPuro will be used in experiments investigating the expression of host genes to better understand mycoplasmal biology and the universal elements of life, and to develop novel therapies for mycoplasmal infections

MiniTn4001PsPuro is a shuttle vector for E coli and *Mycoplasma* spp. Puromycin resistance is a selectable marker for successful transformation of the host. Initial experiments will use MiniTn4001PsPuro to optimize conditions for genetic engineering of *M. genitalium*; these experiments are needed because *M. genitalium* is heretofore poorly characterized as a host for genetic engineering.

We aim at establishing the basic steps for studying the activity and expression level of genes in mycoplasma using genetic manipulation. There is no plasmid system for mycoplasma and genetic manipulation usually involves integrative systems such as the one described by Algire et al., (2009) and therefore will include the plasmid MiniTn4001PsPuro and its derivatives.

Derivatives of MiniTn4001PsPuro will be used in experiments investigating the expression of host genes to better understand mycoplasmal biology and the universal elements of life, and to develop novel therapies for mycoplasmal infections

Although this will be our major platform, we may require other vectors later in the project such as MiniTn4001PsTetM and its derivatives. Genetically modified *Mycoplasma Genitalium* may have antibiotic resistance to puromycin. Other antibiotic markers are not expected to be useful but may be resorted to. They would include Tetracycline, chloramphenicol, Kanamycin and Zeocin. Neither unmodified nor modified organisms will be grown in large quantities.

We will be looking at the expression level of all or any of the genes in the organism. There are no particular ones we will be looking at.

The transposon system we are using is Tn4001. It was originally identified in this paper (http://www.ncbi.nlm.nih.gov/pubmed/6323927). It has been used before by other people to modify *M. genitalium* genomes (e.g this paper http://www.ncbi.nlm.nih.gov/pubmed/19687239, which is also where we got the vectors we are using).

Transposition is (to the extent we care about for this amendment) random so we are not targeting ay specific genes. The genes we will be introducing into the organism will include resistance markers (the ones we currently specify in the amendment), transcription factors from E.coli and perhaps other organisms (e.g. *lacI*, *tetR*, *araC*), and reporter genes (any fluorescent protein, *lacZ* etc).

It is still unclear as to how these integrations will affect the organism however it is expected that virulence and infectivity are more likely to be reduced compared to the wild type.

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. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OSP website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

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:

5. Specify DNA/RNA source (organism the sequence is from), promoter used, nature of insert (genes families are acceptable, e.g. fluorescent proteins), if 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
Antibiotic resistance genes such as TetR	MiniTn4001PsPuro	Spiralin promoter	Standard plasmid collection	Antibiotic resistance	yes	0
Transcription factors such as lacI	MiniTn4001PsPuro	Spiralin promoter	E coli	Transcription factor	yes	0
Reporter genes such as GFP, or lacZ	MiniTn4001PsPuro	Spiralin promoter, inducible promoters such as lac promoter	Jellyfish, synthetic, E coli	Reporter	yes	0

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

7. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

8. 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:

4. Identify cloning/expression/transfection vectors used, recipient bacterial strains used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, 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 strain	Reporter gene?	% viral genes	Type of promoters	Host cell
MiniTn4001PsPuro and its derivatives	E coli K12	Antibiotic resistance	0	Constitutive, inducible	Mycoplasma genitalium

5. 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: https://cabescro.mit.edu/home

Viral Vector type	
Description (Plasmids used, viral vector generation for lentiviral vector)	
Source (vendor / collaborator)	
Packaging cell line(s), if applicable	
Replication competent or incompetent	
Assays for detecting replication competent virus, if applicable	
Pseudotype	
Host range	
Safety feature (e.g. self-inactivating)	
Integrate into genome (yes/no)	
Exposure hazard (e.g. insertional mutagenesis)	
Promoters to be used with viral vector	
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

6. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

a. 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 to register your animal research; If you have CAC protocol no(s), list them here:______________________. The questions below are intended to deal with the use of potentially biohazardous agents in animals/insects or the creation of transgenic animals/insects.

4. 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?

5. 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.

6. 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? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

7. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

8. Describe the steps used to handle and contain spills:

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/animals/plants/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 used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Escherichia coli K-12	MG1655, DH5alpha, EMG2, KL463, XL10, C600, DH10B, BW25113, DH5alpha Pro, MG1655 Pro, RFS289, CJ236, ER2738, ER2267, NEB10beta,	1	CGSC, NEB, ATCC, Thermo, Lucigen, Keith Shearwin, Laub lab, Collins lab, Agilent, expressys, invitrogen, epibio, clontech, Roberto Kolter, real biotech corporation, the Yale E. coli genetic stock center	100mls		Bleach	N

	E. cloni 10G, XL1-GOLD, XL1BLUE, XL1-RED, W3110, NM2, S17, CR63, CR63l, ME5486, transformax EC100D pir, Transformax EC100D pir-116, SURE2, MK01, MK02, ET12567, C600, W1, MC4100, oneshot TOP10, SHuffle-T7, MG1655(DE3), NEB10beta, MG1655 ΔntrBC, MG1655Pro with pLtetO-mf-Lon protease integrated, Transformax EPI3000, DH5alpha F' iq, sbtl3, sbtl4, stellar, HIT DH5-alpha, JM109						
Mycoplasma genitalium	ATCC33530	2	ATCC	100mls		Bleach	Y

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

Organism	Infectious Dose	Route of exposure	Host range	Clinically relevant Drug resistance	Availability of treatment	Special equipment /PPE used (above standard PPE)	Disease state and symptoms of exposure
Mycoplasma genitalium	Unknown	Mucosal contact; injection/ non-intact skin	Humans	Species resistant to beta-lactam; tetracyclines	Macrolides, moxifloxacin	N/A	non-gonococcal urethritis (NGU) i.e. inflammation of the urethra. Symptoms include blood in the urine or semen, burning pain while urinating, discharge from penis, fever, frequent or urgent urination, itching, tenderness or swelling in penis or groin area, abdominal pain, chills, pelvic pain and vaginal discharge

a2. List and describe any other biological material not covered in the tables above:

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

7. Briefly describe experiment and procedures involving use of biological agents?

Growth and transformation of E coli are performed using standard protocols, as described in Current Protocols in Molecular Biology[2]. Growth and modification of M. genitalium follow the protocols described in Algire et al., (2009) *Antimicrobial Agents and Chemotherapy* 53:4429-4432

8. 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:

Transformation will result in puromycin (or if necessary other antibiotic) resistance. No experiments will never add resistance to clinically relevant antibiotics.

9. Will you introduce viable biological material (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 3. Research Description (required): Project 5

Synthetic Biology of Probiotic and Commensal Bacteria

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

The number of microbial cells in the human body outnumbers the number of human cells by 10:1. Collectively, these microbes compose the human microbiome. The microbiome's impact on human physiology and disease pathology has become apparent due to advances in high-throughout DNA sequencing technology and germ-free model organisms, such as gnotobiotic mice. Along with this understanding of its function, we are developing tools to intelligently manipulate the microbiome to treat disease.

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.

5.1) Engineered *E. coli* and *Lactococcus lactis* for the detection of blood in the gastrointestinal tract
The goal of this project is to engineer a strain of *E. coli* that is able to sense gastrointestinal bleeding. Genetic engineering involved testing various genetic parts from distant organisms (*Lactococcus lactis* and enterohemorrhagic *E. coli*) to get a genetic circuit that responds to heme, a component of blood. Testing of responsiveness to blood will be performed both *in vitro* as well as in mouse models of colitis and gastric ulcer. Ultimately, this strain will be paired with CMOS microelectronics for testing both in the lab and in a pig model of bleeding (with collaborators who would be running the pig model).

5.2) Engineering outer membrane vesicles in *Bacteroides spp.*

The goal of this project is to engineer outer membrane vesicles (OMVs) secreted by various species of *Bacteroides*. OMVs are lipid vesicles that contain many bacterial products and have been shown to interact with the host immune system. Our goal is to devise strategies to package non-native proteins into *Bacteroides* OMVs to manipulate the host immune system. These proteins include reporter genes (NanoLuc), model antigens (asparaginase II, ovalbumin) and immunomodulatory proteins (IL-10, IL-22, TGFbeta1). The effects of engineered OMVs will be tested *in vitro* with a variety of mammalian cells lines including MC/9, Caco2 and HT-2. Additionally, OMVs will be tested with primary cell lines derived from wild-type C57BL/6J and transgenic OT-II mice. Finally, engineered strains of *Bacteroides* that secrete modified OMVs will be tested in mouse models of colitis and delayed-type hypersensitivity.

5.3) Caffeine producing *E. coli*
As a proof of concept that expression of bioactive molecules produced in the gut by commensal bacteria can alter behaviors of the carrying organism, we intend to create an *E. coli* strain that produces caffeine. The biosynthetic pathways leading to the synthesis of caffeine will be cloned from *caffea Arabica* (7-methylxanthosine synthase, 7-methylxanthine N-methyltransferase, and 3,7 methylxanthine transferase) and placed under the control of strong inducible promoters such as Plac or Ptet in a plasmid of Lutz and Bujard collection (Lutz and Bujard, 1997). The correct expression of cloned genes will be tracked through HPLC following the appearance of products corresponding to each intermediate state in the caffeine production cascade. We will test production in a variety of media.In order to assess the biological activity of the caffeine produced by our engineered *E. coli* strain, we will colonize streptomycin treated mice with the engineered caffeine producer or a non producing strain and expose groups of mice to the pathway inducer and record their activity as caffeine is known to stimulate hyperactivity by measuring what distance they cover over a given amount of time. Their movements will be tracked by camera.

5.4) A synthetic biology toolkit for the gut commensal *Bacteroides* spp.
In the first (current) generation of microbiome therapy, gut microbes are transferred from healthy donors to sick patients via Fecal Microbiome Transplant (FMT) to establish healthy ecosystems of bacteria. This approach works well to cure diseases resulting from pathogenic organisms, such as *Clostridium difficile*, and it is also being investigated to treat metabolic disease and inflammatory bowel disease (IBD). The second generation of microbiome therapy will use manufactured collections of bacteria in pill form, rather than stool, as a therapeutic. This offers the opportunity to include genetically engineered bacteria as members of the manufactured microbial community. Alternatively, individual strains of genetically engineered bacteria may be fed independently as probiotics and spread their genetic material via conjugation in order to “edit” the microbiome and deliver beneficial genes.

The proposed project aims to investigate whether genetically engineered species from the genus *Bacteroides* are capable of stably colonizing and delivering therapies to the mammalian gut. As a model organism for this genus, we will engineer *Bacteroides thetaiotaomicron VPI-5482 (B. theta). B. theta* will first be genetically manipulated to constitutively express GFP at different levels. This will be accomplished by establishing a collection of *Escherichia coli S17-1 λ pir* clones harboring plasmids with different random sequences in the ribosome binding site preceding the GFP coding region. This collection of *E. coli* strains will be established via standard molecular biology techniques. *Escherichia coli S17-1 λ pir* is a non-pathogenic, attenuated laboratory strain of *E. coli*. The *E. coli* will then be mated with *B. theta* to conjugate their plasmid, which will be integrated into the *B. theta* genome. Subsequently, we will identify *B. theta* clones expressing GFP at different levels via flow cytometry. These B. theta clones will then be used for colonization experiments in gnotobiotic mice. All animal experiments are considered ABL1 since the agents involved are not harmful to humans.

Initial colonization experiments will determine how well engineered *B. theta* colonizes the gut when challenged with wild type *B. theta,* other members of the *Bacteroides* genus, or entire collections of microbiome bacteria derived from specific pathogen-free (SPF) mouse stool. Gnotobiotic mice will be colonized with engineered *B. theta* via oral gavage. 6-7 days later, these mice will be gavaged with a challenge strain or a collection of strains. Mice will be monitored and their feces will be collected for up to a month. To determine colonization efficiency, total DNA will be extracted from feces and subject to qPCR. Flow cytometry will be conducted on engineered *B. theta* isolated from feces to confirm continuous expression of GFP.

If these initial studies are successful, *B. theta* will then be engineered to deliver therapeutic molecules to the mammalian gut. B. theta will express biosynthetic genes to generate the small molecule salicylate. Salicylate is an active metabolite of aspirin and is well appreciated for its anti-inflammatory and analgesic properties (*Acetylsalicylic Acid,* Karsten Schrör, Wiley-VCH, 2012). The genes for salicylate biosynthesis will be identified based on homology to either PchAB from *Pseudomanas aeruginosa* or Irp9 from *Yersinia enterocolitica.* In these organisms, salicylate production is used for iron acquisition and, thus, PchAB and Irp9 are not associated with virulence. The DNA for these genes will be dual codon optimized for *E. coli* and *B. theta* and manufactured via DNA synthesis. No physical contact with *P. aeruginosa* or *Y. enterocolitica* will be necessary. The genes will be cloned into laboratory strains of *E. coli* via standard molecular biology techniques and screened for their efficiency at producing and secreting salicylate *in vitro* via LCMS. The most efficient genes will then be conjugated into *B. theta* as described above. Subsequent to validation of function in *B. theta in vitro,* these *B. theta* clones will be gavaged into mice and screened for *in vivo* production of salicylate as measured via LCMS performed on stool.

Mobile Genetic Elements in Bacteroides

One strategy to stably establish populations of engineered, therapeutic bacteria in the mammalian gut involves the mobilization of payloads from probiotic to endogenous members of the gut flora. Horizontal gene transfer refers to a widespread phenomenon in bacteria whereby genes can be exchanged with distant relatives. This process amplifies evolutionary innovation by allowing the rapid dissemination of beneficial traits throughout a bacterial population. In addition to uptake of environmental DNA, several different classes of mobile genetic elements are involved in horizontal gene transfer, including conjugative plasmid, conjugative transposons and bacteriophage. Although this process is often studied in the context of antibiotic resistance, metabolic genes and other cargo that confer beneficial traits are often traded between bacteria. Likewise, engineered synthetic gene circuits could be loaded onto mobile genetic elements to potentially permit their introduction into natural populations.

The proposed project seeks to investigate conjugative transposons in *Bacteroides* sp. to assess their spread in populations in the murine gut. *Bacteroides thetaiotaomicron* VPI-5842 and the conjugative transposons CtnDOT, CtnERL and Ctn12256 will be used as a model system. CtnDOT and Ctn12256 encode resistance to both tetracycline and erythromycin, whereas CtnERL only encodes resistance to erythromycin[1]. These transposons have been shown to mediate their self-transfer from one strain of *Bacteroides* to another. Initial *in vitro* experiments will investigate the transfer efficiencies and host ranges of these conjugative transposons. The studies will also include other members of the *Bacteroides* genus, including *B. fragilis*, *B. ovatus, B. uniformis*, B. *vulgatus* and *B. caccae*. Resistance markers encoded on the transposons themselves will be used to monitor transfer of the mobile genetic elements. Standard reporter genes (such as GFP or RFP) will be cloned into the transposons to validate the transfer of modified transposons from donors to recipients.

Once movement of these transposons is validated *in vitro*, we will monitor the transfer of these elements *in vivo*, in collaboration with the Fox lab. Gnotobiotic mice will be colonized with individual or a collection of the *Bacteroides* strains listed above and subsequently dosed with *B. thetaiotaomicron* harboring a transposon. Successful transfer will be monitored by collecting feces and performing culture, qPCR and/or flow cytometry assays. If successful, these transfer events will be further monitored in the gut of SPF mice. These initial studies will allow for the establishment of some of the rules that govern gene transfer *in vivo* and will allow for future studies which aim to stably introduce therapeutic gene circuits in the endogenous flora of mammals.

Strains

All strains of *Bacteroides* that will be employed in these studies are non-pathogenic, commensal strains initially isolated from the stool of healthy humans. *Bacteroides* is the most abundant member of the human intestinal flora and has been shown to confer multiple health benefits, including stimulating the immune system[2], alleviating symptoms of colitis[2] and behavioral disorders[3].

Bacteroides strains:

- *Bacteroides thetaiotaomicron* VPI-5482
- *Bacteroides fragilis* NCTC 9343
- *Bacteroides vulgatus* ATCC 8482
- *Bacteroides ovatus* ATCC 8483
- *Bacteroides caccae* ATCC 43185
- *Bacteroides uniformis* ATCC 8492

B. fragilis is an opportunistic pathogen that can cause disease in humans who experience intestinal trauma. However, there are no known invasive factors that allow *B. fragilis* to cause disease without a breach in intestinal integrity.

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. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OSP website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

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
NanoLuc	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Geneblock	Luminescent reporter gene	Yes	0
mIL-10	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Mouse	Anti-inflammatory protein	Yes	0
mIL-22	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Mouse	Anti-inflammatory protein	Yes	0
OMV-Association Tag	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Bacteroides	Secretion Tag	Yes	0
Cas9	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	S. pyogenes	CRISPR-Component	Yes	0
dCas9	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	S. pyogenes	CRISPR-Component	Yes	0
Guide RNA	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Geneblock	CRISPR-Component	No	0
Antigens (Ova, MOG)	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Chicken/Mouse	Antigen	Yes	0
Alkaline Phosphatase	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Bacteria, Cow, Human	Alkaline Phosphatase	Yes	0
dsbAB	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1),	E. coli	Protein Chaperones	Yes	0

		P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)				
LL37	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Human	Antimicrobial peptides	Yes	0
REG3g	pNBU1, pNBU2	P(Rha), P(L23R), P(BT0268), P(BT3324), P(1), P(cfxA), P(cfiA), P(cepA), P(BfE6), P(BT1311)	Human	Antimicrobial peptides	Yes	0
hrtR	p15a, ColE1	hrtR	L. lactis	Transcriptional repressor	Yes	0
luxCDABE	ColE1	PL(hrtO), P(lux), P(phsA)	Addgene vector	Luminescent reporter operon	Yes	0
ChuA	p15a, ColE1	P(J23107)	E. coli	Heme importer	Yes	0
LuxR	ColE1	P(K176009)	Addgene vector	Transcription factor	Yes	0
ThsRS	p15a, ColE1	P(ThsRS)	Addgene vector	Two-component system	Yes	0
Enzymes from caffeine pathway	Lutz and Bujard collection[1.]	pLac, pTET	*caffea Arabica*	Enzymes	Yes	0

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

2. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

3. 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 used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, 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 strain	Reporter gene?	% viral genes	promoters	Host cell
pNBU1, pNBU2	Bacteroides thetaiotaomicron, B. fragilis, B. ovatus, B. vulgatus	Y	0	Constitutive and inducible	Bacteroides thetaiotaomicron, B. fragilis, B. ovatus, B. vulgatus

pZA1	E. coli Nissle 1917	N	0	Constitutive	E. coli Nissle 1917
pZE1, pZE2, pZE3	E. coli Nissle 1917	N	0	Constitutive	E. coli Nissle 1917
pBR322	E coli K12	Y	0	Constitutive	E. coli K12

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: https://cabescro.mit.edu/home

Viral Vector type	
Description (Plasmids used, viral vector generation for lentiviral vector)	
Source (vendor / collaborator)	
Packaging cell line(s), if applicable	
Replication competent or incompetent	
Assays for detecting replication competent virus, if applicable	
Pseudotype	
Host range	
Safety feature (e.g. self-inactivating)	
Integrate into genome (yes/no)	
Exposure hazard (e.g. insertional mutagenesis)	
Promoters to be used with viral vector	
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

7. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

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 to register your animal research; If you have CAC protocol no(s), list them here: **1215-113-18, 0915-092-18**. 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?

1215-113-18
Our idea is to engineer orally administered probiotic yeast S. boulardii that produce fusion proteins, composed of luciferase fused to Fc fragments, near the gut epithelium to be taken up systemically via the FcRn pathway. Our proposed platform would enable the oral administration of freeze-dried engineered probiotics for systemic protein delivery – this transformative system would yield an extensible heat-stable, and low cost formulation ideal for low-resource settings. We will express the GLuc-Fc fusion protein using a pTDH3 or pTEF2 constitutive promoter on a pRS30x standard cloning vector. Secretion will be achieved by fusing of the protein to a secretion leader. pRS30x plasmids are integrative (no yeast replication origin) and differ by their selective marker (HIS, LEU, TRP or URA). Selection markers used are the auxotrophic markers (URA, HIS, LEU, TRP). Neither of these proteins by themselves confers any level of pathogenicity.

0915-092-18:
We will introduce recombinant organisms, including engineered E. coli, B. thetaiotaomicron, B. fragilis, B. ovatus and B. vulgatus strains, as well as the probiotic yeast S. boulardii into the mouse gastrointestinal tract. The objective is to test genetically engineered strains for their ability to detect gut inflammation and/or secrete anti-inflammatory proteins, for their ability to act as as next-generation of inflammation bowel disease sensors and therapeutics. Organisms will be introduced by oral gavage. Recipient species includes Balb/c mice, SJL mice, and C57BL/6 mice. No engineered bacterial strains are known to have or will acquire virulence. The bacteria may have display resistance to common laboratory antibiotics such as kanamycin, or erythromycin, but not to clinically relevant drugs

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? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

1. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

2. Describe the steps used to handle and contain spills:

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/animals/plants/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 used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Bacteroides eggerthii	VPI T5-42B-1 (ATCC27754)	2	ATCC	100mls		Bleach	Y
Bacteroides acidifaciens	mmF857	2	Mouse isolate MIT	100mls		Bleach	Y
Bacteroides ASF519	mmF835	2	Mouse isolate MIT	100mls		Bleach	Y
Bacteroides caccae	ATCC 43185	2	ATCC	100mls		Bleach	Y
Bacteroides fragilis	NCTC 9343	2	ATCC	100mls		Bleach	Y
Bacteroides ovatus	NCTC 11153, ATCC 8483	2	ATCC	100mls		Bleach	Y
Bacteroides thetaiotaomicron	VPI-5482, NCTC 9343, HSP40	2	ATCC	100mls		Bleach	Y
Bacteroides uniformis	ATCC 8492	2	ATCC	100mls		Bleach	Y
Bacteroides vulgatus	ATCC 8482, mmF837	2	ATCC, mouse isolate MIT	100mls		Bleach	Y
Bacteroides vulgatus	ATCC 8482	2	ATCC	100mls		Bleach	Y
Enterobacter aerogenes	planned	2	ATCC	100mls		Bleach	Y
Enterobacter cloacae	1000654 (NDM-1)	2	ATCC	100mls		Bleach	Y
Enterococcus faecalis	Ef1 – Ef15	2	ATCC, Mia Liebermann (Fox lab)	100mls		Bleach	Y
Escherichia coli K-12	MG1655, DH5alpha, EMG2, KL463, XL10, C600, DH10B, BW25113, DH5alpha Pro, MG1655 Pro, RFS289, CJ236, ER2738, ER2267, NEB10beta, E. cloni 10G, XL1-GOLD, XL1BLUE, XL1-RED, W3110, NM2, S17, CR63, CR63l, ME5486, transformax EC100D pir, Transformax EC100D pir-116, SURE2, MK01, MK02, ET12567, C600, W1, MC4100, oneshot	1	CGSC, NEB, ATCC, Thermo, Lucigen, Keith Shearwin, Laub lab, Collins lab, Agilent, expressys, invitrogen, epibio, clontech, Roberto Kolter, real biotech corporation, the Yale E. coli genetic stock center	100mls		Bleach	N

	TOP10, SHuffle-T7, MG1655(DE3), NEB10beta, MG1655 ΔntrBC, MG1655Pro with pLtetO-mf-Lon protease integrated, Transformax EPI3000, DH5alpha F' iq, sbtl3, sbtl4, stellar, HIT DH5-alpha, JM109						
Escherichia coli (other)	ATCC 11775, ATCC 700973, ATCC 23503, ATCC 23511, GUE, 5649, RKI, BAA-201 (TEM-3), Nissle 1917, HS, ECOR collection (ECOR1-70)	2	ATCC, P. Nordmann, STEC(http://shigatox.net/new/reference-strains/ecor.html)	100mls		Bleach	Y
Escherichia coli B	BL21, BL21(DE3), Rosetta, Rosetta(DE3), BL21AI, BB, REL606, BL21(DE3)/pLysS, SHuffle express T7, Express Iq	1	ATCC, NEB, Lucigen, Invitrogen, TKL	100mls		Bleach	N
Escherichia coli C	C-1, C-1792 , C-236 , C-367 , C-368	1	Ian Molineux, ATCC, TKL	100mls		Bleach	N
Escherichia coli	rcF471-6, rcF479, rcF513-9	1	Mouse fecal isolates	100mls		Bleach	N
Proteus mirabilis	rcF520-22	2	Mouse fecal isolates	100mls		Bleach	Y
Lactobacillus plantarum	WCFS1	1	ATCC	100mls		Bleach	N
lactococcus lactis	planned	1	ATCC	100mls		Bleach	N
Parabacteroides distastonis	mmF840, NCTC11152	2	Mouse isolate (MIT animal facility), ATCC	100mls		Bleach	Y
saccharomyces cerevisiae	BY4741	1	TKL	100mls		Bleach	N

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

Organism	**Infectious Dose**	**Route of exposure**	**Host range**	**Clinically relevant Drug resistance**	**Availability of treatment**	**Special equipment /PPE used (above standard PPE)**	**Disease state and symptoms of exposure**

Bacteroides thetaiotaomicron; B. ovatus; B. uniformis; Parabacterioides distastonis; Bacteroides caccae; B. eggerthii;	Unknown	Mucosal contact; injection/ non-intact skin	Humans, dogs, cats, and other animals		Metronidazole, imipenem, and amoxicillin seem to be effective against B. fragitis and B. thetaiotaomicron [16]. Studies using a rat model have shown that pretreatment using oral vancomycin/imipenem resulted in undetectable levels of Bacteroides spp	N/A	Bacteroides spp. represent an important anaerobic bacterial genus associated with human infections[3]. In combination with other facultative/strict anaerobes, they are responsible for the majority of localized abscesses within the cranium, thorax, peritoneum, liver, and female genital tract[4,8]. They can cause pulmonary abscesses when naturally-occurring oropharangeal Bacteroides and closely related genera are aspirated into the lung[8]. These taxa can lead to many types of diseases, some of which can be fatal, including noma (cancrum oris), human apical periodontitis, endocarditis, pelvic inflammatory disease, suppurative thrombophelebitis, and wound infections[4,6,9]. Organisms from oral flora also have a role in dental abscesses and infectivity of human bites. Bacteroides fragilis is the most common opportunistic pathogen of Bacteroides spp.[1,4]. Spread to bloodstream (bacteremia) is more common for B. fragilis than any other anaerobe[4]. Deep pain and tenderness below the diaphragm are typical of B. fragilis infection. Widespread intra-abdominal abscesses may be associated with fever and abdominal pain.
ENTEROCOCCUS FAECALIS	Unknown	Ingestion; mucosal contact; injection/ non-intact skin	Humans	Strains resistant to β-lactams, aminoglycosides and, increasingly, vancomycin have been described[2,4].	Most strains remain susceptible to penicillin, ampicillin, and vancomycin.		Enterococci can cause urinary tract, wound, and soft tissue infections[2,4]. They are also associated with bacteremia which can

				Strains have also been identified which carry genetic elements conferring resistance to chloramphenicol, tetracyclines, macrolides, lincosamides, quinolones, and streptogramins(2).			lead to endocarditis in previously damaged cardiac valves(4). E. faecalis is the most frequent species isolated from human intestine samples (80-90%), E. faecium accounts for 5-10% of isolates(1
ENTEROBACTER AEROGENES and cloacae	Unknown	Ingestion; injection/ non-intact skin	Humans	Enterobacter spp. are resistant to ampicillin; first- and second- generation cephalosporins(7); and cephalothin(	Most Enterobacter spp. are susceptible to cefepime(7), aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole(8). Tigecycline has been shown effective in vitro		Enterobacter spp., particularly E. aerogenes and E. cloacae, have been associated with nosocomial outbreaks, and are considered opportunistic pathogens(1,5). Enterobacter spp. can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections(6,7). In addition, Enterobacter spp. have been noted in intravascular device-related infections, and surgical site infections (primarily postoperative or related to devices such as biliary stents)(7). Many species can cause extra-intestinal infections(6), for example, Enterobacter sakazakii, has been associated with brain abscesses in infants and with meningitis(3,7). Mortality rates for bacterial meningitis range from 40-80%(5).

a2. List and describe any other biological material not covered in the tables above:

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

1. Briefly describe experiment and procedures involving use of biological agents?

Bacterial growth and transformation will be done using standard protocols, such as those described in Current Protocols in Molecular Biology[2].

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:

Bacteria will gain antibiotic resistance as well as the ability to synthesize various compounds as described.

3. Will you introduce viable biological material (microorganisms or cells) into animals?

Yes ☒ No ☐ If so:

a) Please describe:

1215-113-18

Our idea is to engineer orally administered probiotic yeast S. boulardii that produce fusion proteins, composed of luciferase fused to Fc fragments, near the gut epithelium to be taken up systemically via the FcRn pathway. Our proposed platform would enable the oral administration of freeze-dried engineered probiotics for systemic protein delivery – this transformative system would yield an extensible heat-stable, and low cost formulation ideal for low-resource settings. We will express the GLuc-Fc fusion protein using a pTDH3 or pTEF2 constitutive promoter on a pRS30x standard cloning vector. Secretion will be achieved by fusing of the protein to a secretion leader. pRS30x plasmids are integrative (no yeast replication origin) and differ by their selective marker (HIS, LEU, TRP or URA). Selection markers used are the auxotrophic markers (URA, HIS, LEU, TRP). Neither of these proteins by themselves confers any level of pathogenicity.

0915-092-18:

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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: **0915-092-18; 1215-113-18;**

Section 3. Research Description (required): Project 6: Genetically encoded state machines design operation in *E. coli*.

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

Bacteria sense chemicals in their environment, and respond by modulating their gene expression. This ability can be engineered to make living sensors for a variety of uses in the environment and medicine. In these experiments we are creating a basic platform into which sensing and responding circuits can be plugged. This will allow synthetic biologists to bypass creating each system from scratch.

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.

The goal is to engineer logic in *Escherichia Coli* (*E. coli*). We are trying to engineer the way that *E. coli* responds to chemical signals. For example, if chemical A and chemical B are in the environment, then we want *E. coli* to produce protein C. This is one example type of logical statement that we are trying to engineer. We aren't building sensors for input chemicals, nor are we testing new output proteins, we are just developing the "biological hardware" that lets us map input chemicals to output proteins. The "biological hardware" is DNA recombination systems. These systems are composed of recombinases, which target two specific (~50 nucleotide long) DNA sites and cause recombination between them. We leverage these controlled recombinase-based recombination events to map input chemicals to outputs. The recombinase systems, which are typically from bacteriophage, are introduced into *E. coli* via recombinant DNA on plasmids. The plasmids have standard antibiotic selection markers (i.e. chloramphenicol, kanamycin, streptomycin, spectinomycin, carbenicillin, ampicillin, tetracycline, and gentamicin resistance genes). The chemical inputs that we use to test our systems are canonical inducers used in synthetic biology: anhydrotetracycline (ATc), diacetylphloroglucinol (DAPG), acetyl homoserine lactone (AHL), Isopropyl β-D-1-thiogalactopyranoside (IPTG), and sugars like arabinose (Ara). The inducers are sensed via inducible promoter systems, introduced into *E. coli* via recombinant DNA on plasmids with the same antibiotic resistance markers as mentioned above. Lastly, the protein outputs that we used to test our engineered systems are fluorescent proteins (e.g. GFP, RFP, and BFP). These are also introduced into *E. coli* via recombinant DNA on plasmids with the same antibiotic resistance markers as mentioned above.

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. For information on which section of the NIH Guidelines your experiments fall under, please see the NIH OSP website: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines.

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:

4. Specify DNA/RNA source (organism the sequence is from), promoter used, nature of insert (genes families are acceptable, e.g. fluorescent proteins), if 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
Antibiotic resistance genes, such as kanR	Vectors from the Lutz and Bujard collection[1].	Native, constitutive	bacteria	Antibiotic resistance genes	Y	0
Fluorescent proteins such as GFP	Vectors from the Lutz and Bujard collection[1].	Constitutive, such as prod, PBT3763	Jellyfish, synthetic	Fluorescent proteins	Y	0
Recombin-ases such as bxb	Vectors from the Lutz and Bujard collection[1].	Inducible promoters including PBAD, PluxS, Ptet, pRHA	Bacteria, bacteriophage, synthetic	recombinases	Y	<10%

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

6. Do any sequences code for toxins not covered in (2) above? Yes ☐ No ☒; If yes, please specify.

7. 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:

3. Identify cloning/expression/transfection vectors used, recipient bacterial strains used to propagate the vector, and recipient host cell (bacteria, yeast, murine or human cells, 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 strain	Reporter gene?	% viral genes	Type of promoters	Host cell
Vectors from the Lutz and Bujard collection[1.]	E coli K12	Fluorescent proteins	0	Constitutive for antibiotic resistance and fluorescent reporters, inducible for recombinases	E coli K12

4. 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: https://cabescro.mit.edu/home

Viral Vector type	
Description (Plasmids used, viral vector generation for lentiviral vector)	
Source (vendor / collaborator)	
Packaging cell line(s), if applicable	
Replication competent or incompetent	
Assays for detecting replication competent virus, if applicable	
Pseudotype	
Host range	
Safety feature (e.g. self-inactivating)	
Integrate into genome (yes/no)	
Exposure hazard (e.g. insertional mutagenesis)	
Promoters to be used with viral vector	
Inserts to be used with viral vector[1,2]	

[1]For Cas9 expression in viral vectors, please indicate whether the promoter driving it is inducible or constitutively active.

[2]List all oncogenes that will be overexpressed and all tumor-suppressors that will be knocked down using viral vectors.

8. If using nanoparticles for the delivery of recombinant DNA/RNA, fill out the table below.

Nanoparticle description	
Able to enter cells? (yes/no)	
Exposure hazard	

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 to register your animal research; If you have CAC protocol no(s), list them here:_____________________. 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 ☐

   b. 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? Yes ☐ No ☐

If yes, an SOP is required for this work. Contact the Biosafety Program for assistant and:

1. Identify culture room, biological agent, recombinant modifications, and type of equipment used for large-scale culture growth and handling:

2. Describe the steps used to handle and contain spills:

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.)

a1. Agent identification. List biological agent(s)/microorganism(s) genus/animals/plants/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 used	Max Concentration used	Disinfectant (s) used	Can this cause disease in any population? (Y/N)
Escherichia coli K-12	MG1655, DH5alpha, EMG2, KL463, XL10, C600, DH10B, BW25113, DH5alpha Pro, MG1655 Pro, RFS289, CJ236, ER2738, ER2267, NEB10beta, E. cloni 10G, XL1-GOLD, XL1BLUE, XL1-RED, W3110, NM2, S17, CR63, CR63l, ME5486, transformax EC100D pir, Transformax EC100D pir-116, SURE2, MK01, MK02, ET12567, C600, W1, MC4100, oneshot TOP10, SHuffle-T7, MG1655(DE3), NEB10beta, MG1655 ΔntrBC, MG1655Pro with pLtetO-mf-Lon protease integrated, Transformax EPI3000, DH5alpha F' iq, sbtl3, sbtl4, stellar,	1	CGSC, NEB, ATCC, Thermo, Lucigen, Keith Shearwin, Laub lab, Collins lab, Agilent, expressys, invitrogen, epibio, clontech, Roberto Kolter, real biotech corporation, the Yale E. coli genetic stock center	100mls		Bleach	N

	HIT DH5-alpha, JM109						

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

Organism	Infectious Dose	Route of exposure	Host range	Clinically relevant Drug resistance	Availability of treatment	Special equipment /PPE used (above standard PPE)	Disease state and symptoms of exposure

a2. List and describe any other biological material not covered in the tables above:

a3. Are any of the biological agents used or stored in your laboratory select agents? Check here to verify (https://www.selectagents.gov/selectagentsandtoxinslist.html). Yes ☐ No ☒ If yes, which ones?

b. Experimental Procedures:

1. Briefly describe experiment and procedures involving use of biological agents?

Bacterial growth and transformation will be done as described in Current Protocol in Molecular Biology (Online ISBN: 9780471142720).

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:

Antibiotic resistance, fluorescence

3. Will you introduce viable biological material (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.

c. Human material used (check all that apply):

x	Established human cell lines		Human blood, serum, plasma, blood products, or components
	Primary human cell lines		Human bodily fluids

	Unfixed human tissues or organs		Cells, tissues, or organs containing HIV or HBV

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/2009-guidelines.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.

hES cell line name	NIH registry #	Source (vendor / collaborator / Institution)

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

6. **Do you have human embryonic stem cells stored that are no longer used in your laboratory?**
   Yes ☐ No ☐ If yes, please explain.

7. **Will you 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.

8. **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 ☐

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

10. **Do you plan to inject or transplant iPS cells in any animal, animal embryo or at any stage or prenatal development where the 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 handled 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. 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.
2. Please complete a Standard Operation Procedure (SOP) template and read the MIT EHS Select Agent Toxin Control and Containment Program: Purchasing, Inventory, Shipping and Receiving Procedures SOP. Both 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	Functional toxin?	Source (vendor)	LD_{50} (mg/kg)	Toxicity mechanism (e.g. B subunit mediates receptor binding and cell entry, A subunit carries toxic activity) and symptoms of exposure
Diphtheria *					
Cholera					
Shiga					
Aflatoxin					
Chlorotoxin					

* Routine vaccination for diphtheria [with Td or Tdap vaccine] protects against diphtheria toxin and is advised for all adults every 10 years. Personnel working with diphtheria toxin are well advised to keep up to date on this health maintenance recommendation.

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. Please complete all sub-sections 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 the strains used attenuated?
Yes ☐ No ☒ **If so, explain:** no strains used

c. 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.

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 (c)?
Yes ☐ No ☒ **If so explain:**

e. If your research does not 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):

   For pathogens, please see section 6.

   For viral vectors, please see section 5.

   BL1 bacterial strains (e.g. *E. coli* K-12) used in the lab are non-infectious to humans. They are all safely used following BL1 practices and procedures.

   Human cell lines are covered in the Exposure Control Plan (please see document for more detail) and follow BL2 practices and procedures

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.

X	Lab Coat	X	Gloves
	Safety glasses		Safety Goggles
	Face shield (please specify procedure):		Other (Please describe PPE and procedure):

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

Liquid waste decontamination	
X	10% final concentration of household bleach (0.5% NaOCl), 20 minute contact time
	Wescodyne (1%), 20 minute contact time
	Autoclave
	Other (Please describe rationale):
Work surfaces and equipment decontamination	
X	10% final concentration of household bleach (0.5% NaOCl)

X	70% ethanol (Note: ethanol is not an appropriate disinfectant for work involving human materials)
X	EPA approved product such as Sklar, Lysol spray, PREempt, etc. (Please list product(s)): Pre-Empt
	Other (Please describe):

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

X	Solid waste	Placed in EHS provided biowaste boxes for disposal
	Solid Waste	Autoclave (please describe rationale):
X	Animal or human tissue	Placed in EHS provided biowaste boxes dedicated for incineration
X	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).

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

Footnotes:

1. Vectors from the Lutz and Bujard collection (https://www.ncbi.nlm.nih.gov/pubmed/9092630).
2. Current Protocols on Molecular Biology (Online ISBN: 9780471142720).