For office use only Next Renewal Due: 01/2007 Next Rewrite Due: 01/2009	304

Massachusetts Institute of Technology
Committee on Assessment of Biohazards

Biological Research Registration Form

Instructions:

This form is available at: http://web.mit.edu/environment/ehs/rdna.html
Please download and save this form to your computer. When completing this document please retain the format as nearly as possible and answer questions thoroughly. Complete the appropriate sections as outlined below. If the research involves use of human materials, you will also need to have an "Exposure Control Plan" in place. More details are provided in Section 6.
Required. Every Principal Investigator must complete the following:

- Section 1 (General Information)
- Section 2 (Laboratory Information)
- Section 3 (Project Description)
- Section 9 (Certification and Signatures).

Research Specific Sections. Complete the following sections if they are applicable to your research as indicated below.

- Section 4 for research involving recombinant DNA technologies.
- Section 5 for research involving viable microorganisms or viruses.
- Section 6 for research involving human materials including human tissues and human derived cell lines. (Note: You will need COUHES approval for most research involving human materials, and you should contact COUHES, Judith Medeiros-Adams, Executive Secretary, Human Subjects Committee at 3-6787.)
- Section 7 for research requiring support from MIT Occupational Medicine Clinicians
- Section 8 for research involving the listed toxins.

When you have completed the form, you need to email the completed form to your BSP contact or to Biosafety@mit.edu. Print and sign Section 9, the Certification and Signature page and mail to Biosafety Program, N52-496.

Section 1. General Information:

a. Project Registration No. 304 (leave blank if new project) Date: Nov 1, 2006

b. Biosafety Level (indicate all that apply) exempt BL1 [BL2] BL2+ BL3

c. Project Title: **rDNA studies in the Schauer lab**

d. Principal Investigator: **David Schauer**

Department: **BE** Campus Address: **56-787B**

Office Phone: **3-8113** Lab Phone: **3-7212**
Fax: **8-0225** email: **schauer@mit.edu**

e. Laboratory EHS Representative: **Katherine Schlieper**

Phone: **3-7212** email: **kschliep@mit.edu**

f. Please attach a list of all laboratory personnel working on this project at MIT, to include faculty, technical staff, graduate students, UROPS, etc.

Section 2. Laboratory Information:

a. Is this project part of a course or teaching lab? YES_____ NO__XX__

b. 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.

		Check box if applicable			
Room Number	Biosafety Level	BSC	Warm/Cold Room	Equipment Room	Human Materials used
56-786	2				
56-765	2			XX	
56-773	2	XX			XX
56-779	2		XX		

c. Will radioactive materials be used? Yes__X_ NO___ **Radiation Protocol No. __BE-G_____**

d. Will animals be used for this project? Yes_X__ NO___ **CAC Protocol No.(s)** 0204-012-07 & 0705-047-08

e. Will mixed waste be generated (radioactive/biological or chemical/biological)? YES__X__ NO_____
If yes, please indicate how you will inactivate the biological component of the mixed waste in the box below.

All will be lysed with detergent before disposal as radioactive waste. As approved by the Radiation Protection Office.

(For information on waste management, please see: http://web.mit.edu/environment/ehs/waste.html. If you have questions, contact EHS at 2-3477).

Section 3. Project Description:

Outline the overall goal(s) of the project 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. Please identify and discuss the health and safety risks associated with this research.

Our studies on microbial pathogenesis focus on (1) naturally occurring murine bacterial pathogens and (2) mechanisms of inflammation and epithelial hyperproliferation in the gastrointestinal tract. Our research is relevant for inflammatory bowel disease, hepatitis, and increased cancer risk associated with these conditions.

The majority of the rDNA made in this lab will be used for sequencing. Other recombinant work will include the use of a antibiotic-resistance marker cassettes to insertionally inactivate bacterial genes of interest. These isogenic bacteria will be characterized in cell culture and in vivo. Stx1 and Stx2 (Shiga toxin genes) carried on bacteriophage will be used to transduce strains of *Escherichia coli* and *Citrobacter rodentium* to convert them to toxigenic strains. These strains will also be characterized in cell culture and in vivo. In some cases, but never for select agent toxins including Stx1 and Stx2, bacterial genes will be cloned for purposes of expression, rather than sequencing. Some of these genes include adhesins and toxins from diarrhea causing strains of Enterobacteriaceae, as well as from *Helicobacter* species and *Campylobacter* species.

Section 4. Use of rDNA:

(Please complete this section if you use or generate recombinant microorganisms, cells, animals, etc.)

a. Source of Gene, Insert or Clone:

Specify DNA/RNA source (or probe), nature of insert, is a protein expressed, and percent of any viral genome in construct:

Cholorophenicol cassette--- from *C. jejuni* -- protein is expressed
***Helicobacter* DNA --*Helicobacte*r spp. --possible protein expression**
***Citrobacter* DNA --*Citrobacter* spp. -- possible protein expression**

1. **STX1 and STX2 --*E. coli* lysogenic phage --protein is expressed**

1. Do any sequences code for toxins? If yes, please specify. **Yes, STX1, STX2, and CDT.** LD50 for CDT is unknown. LD50 in rabbits, Stx1 is 20ng/kg and Stx2 is 900 ng/kg**. STX originates on the bacteriophage and will be used only in this naturally occurring form; no functional Stx clones will ever be generated.**

Is the DNA source from a USDA-regulated plant, animal or insect? 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) **No**

b. Vectors and Host Cells:

1. Identify cloning/expression/transfection vectors used, recipient bacterial strains, and recipient host cell lines (human, mouse, plant, etc.). Provide a restriction map of vector unless this is a commercially available vector. If commercially available, please indicate vendor. Describe the location and type of promoters and other control sequences and percent of any viral genome in construct.

Vectors -- pGEM T-easy vector system 1 – Promega Catalog # A1360
Topo TA Cloning Kit -- Invitrogen Catalog # 45-0641
PBluescript -- from our frozen collection (sequence on the web.)
Recipient bacterial strains-- *E. coli DH5a,E. coli XL1-Blue, E. coli SOLR, E. coli SCS110,*
Citrobacter rodentium

2. If using viral vectors, indicate packaging cell lines and assay system used to measure helper virus titre or titre of replication competent virus (background) generated. Include host range of packaged viral vector. (If using retroviral or lentiviral vectors additional requirements apply. Please see MIT CAB policy at the following URL: http://web.mit.edu/cab/policies.html

NO viral vectors are being used.

c. Use of Recombinant DNA in Animals, Plants or Insects:

1. Please describe how recombinant DNA will be used in animals, plants or insects.

Bacteria carrying the rDNA will be oral gavaged into mice for infection studies.

2. If transgenic or "knockout" animals, plants or insects will be generated or used in the project, please note that and provide information on the injected gene and vector as well as the recipient animal, plant or insect strain.

To be inoculated with any isogenic mutant bacteria:

Rag2 KO	immunodeficient
SCID	immunodeficient
TCR alpha KO	immunocompromised
TCR beta KO	immunocompromised
Min Mice (Apc gene mutation)	model for colorectal cancer
GPT Delta transgenic mice	reporter gene for in vivo mutagenesis assays
IRAK-M KO	immunocompromised
IL-10 KO	immunocompromised
Rag2 KO Min Compound mutant	immunocompromised, model for colorectal cancer

What is the expected phenotype of the animal, plant or insect (for example, immunodeficient, enhanced disease susceptibility, early disease onset or resistance, etc.)? see above

d. Large-Scale Research:

Do experiments involve growth of more than 10 liters of culture at a time? If YES, identify culture room and type of equipment used for large-scale culture growth and handling. **No**

Section 5. Biological Agent Use:

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

a. Agent identification. List biological agent(s)/microorganism(s) (e.g., *H. pylori*, EBV, *E.coli* 0157, B.subtilis, S. cerevisae, etc.) and recommended Biosafety Level (CDC):

Helicobacter* spp., *Citrobacter* spp., *Campylobacter spp. **all BL-2**

Diarrhea-causing strains of *Escherichia coli* (i.e. food- and waterborne pathogens) all BL2

***Escherichia coli* rDNA work is all done with common cloning strains of E.coli, which are BL1**

b. Agent hazard. Is the agent infectious to humans (Y __X__ N____), animals (Y X __ N____) or plants (Y____N __X__)? **(If the answer is no to all, skip the remainder of Part 5.)**

1. Source of infectious agent (e.g., new isolate from human tissue, blood, animal, tissue culture, another laboratory, ATCC, etc.): **Pure culture of bacteria obtained either from ATCC or other sources**

2. Host range: Can this agent infect humans? (If so, provide infectious dose information, when known.) *Campylobacter spp.* and *E. coli*, plus some *Helicobacter spp.* can infect humans. *Citrobacter rodentium* is infectious for rodents, no evidence of infection in humans

c. Experimental Procedures:

1. Describe procedures involving use of infectious agent (indicate culture volume, maximum concentration). How and at what stage of the experiment is the infectious agent inactivated or lysed?

Bacteria will be grown on plates and in broth (less than 500 mL)
DNA will be isolated (plasmic or genomic)
Transformation will be by high-voltage electroporation
Isolate proteins (lysis, metal affinity chromatography)
Inoculate into cultured cells and/or mice
Cultures and cell lines will be lysed at the end of the experiment
Tissues will be collected from mice at necropsy

Will experiments result in acquisition of new characteristics such as enhanced virulence, infectivity, drug resistance, or change in host range? If so, explain: *Citrobacter rodentium* with STX1 or STX2 may cause mice to show signs of EHEC infection. CDT in *E. coli* will cause cellular distention in cell lines.

d. Safety Procedures:

1. Outline protective equipment required to minimize exposure of laboratory personnel during all procedures requiring handling or manipulation of infectious agent:

Please refer to Schauer Lab exposure control plan. Same precautions as with human materials will be taken

2. Outline procedures for decontamination of work surfaces, instruments, equipment, liquid containing infectious materials and glassware:

Please see exposure control plan. The same precautions will be in effect.

3. Outline disposal/decontamination procedures for contaminated sharps, contaminated solid waste, tissues, pipette tips, etc. Please refer to exposure control plan. Same precautions will apply here.

Section 6. Use of Human Source Material:

(Please complete this section if you work with human source material, including human tissues and human cell lines.)

a. List the source and types of human material (i.e., blood, bone, sputum, cell culture). For tissue culture, list cell types and names. Please include where you plan to obtain the material:

List of Human Cell Lines Used in Conjunction with Schauer 304

Catalog No.	***Description***
CCL-2	***HeLa (epitheloid carcinoma, cervix)***
CCL-23	***Hep-2 (epidermoid carcinoma, larynx)***
CCL-248	***T84 (epithelial colonic carcinoma; lung metastasis)***
CRL-1573	***293 (adenovirus-transformed kidney epithelial)***
CRL-1739	***AGS (gastric adenocarcinoma, stomach)***
CRL-10741	***C3A or HepG2/C3A (hepatoblastoma)***
HTB-37	***Caco-2 (Adenocarcinoma, colon)***
CRL-8015	***TK-6 (lymphoblast, thymidine kinase heterozygote)***
TIB-202	***THP-1 (acute monocytic leukemia, monocyte)***
HTB-38	***HT-29 (colorectal adenocarcinoma, colon)***

b. Has the material been treated prior to use in the lab (such as formalin fixing or heat treatment)? Please describe: No

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

YES X___ NO _____ N/A _____

Note: Use of human source material requires the Principal Investigator to comply with all applicable facets of the OSHA Blood Bourne Pathogen Program at MIT, including personnel training and filing an Exposure Control Plan (ECP) with the MIT Biosafety Program. Call 3-1740 with questions. Forms can be downloaded from the following website:

(http://web.mit.edu/environment/environmental/ehs_services/ehs_areas/biosafety/forms/index.html)

d. In order to use human materials/cells/tissue except for established human cell lines available from commercial sources, you may need prior approval from the MIT Committee on the Use of Humans as Experimental Subjects (COUHES). Contact COUHES or EHS for help in determining whether you need COUHES approval. Please include below your approval number, if applicable.

Is COUHES needed? YES___NO____ COUHES Approval No. EXEMPTION # 0411000971

COUHES committee may be contacted at 3-6787. http://web.mit.edu/committees/couhes/index.shtml

Section 7. OCCUPATIONAL MEDICINE. NOT APPLICABLE; group is enrolled in Bloodborne Pathogen program.

Some research protocols rated as Biosafety Level 2 or greater may involve a need for services from the Occupational Medicine clinicians at the MIT Medical Department. If there is a need for the services listed below, check the appropriate box. If you are not sure what your needs are for medical clearance and/or surveillance, contact the Occupational Medicine staff at MIT Medical at 617-253-8552.

- ❑ **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.** 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:_____
  Note: For work with Human Materials or Bloodborne Pathogens, Hepatitis B vaccinations are made available as part of the Bloodborne Pathogen Program.

- ❑ **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 8. Use of Select Agent Toxins of Biological Origin NOT APPLICABLE

Do you plan to store or use any of the following toxins? If so, please indicate which one(s) and the approximate maximum amount stored at any time in your laboratory. (**Note: Lock boxes must be used for storage of any toxins listed below. If you need a toxin lock box, contact BSP at 2-3477 and one will be provided at no cost.**)

HHS Toxins

Toxin	Regulatory Threshold Quantity Requiring CDC certificate of registration	Your maximum quantity
Abrin	100 mg	
Conotoxins	100 mg	
Diacetoxyscirpenol (DAS)	1000 mg	
Ricin	100 mg	
Saxitoxin	100 mg	
Tetrodotoxin	100 mg	
Shiga-like ribosome inactivating proteins	100 mg	

Overlap Toxins

Toxin	Regulatory Threshold Quantity Requiring CDC or USDA certificate of registration	Your maximum quantity
Botulinum neurotoxins	0.5 mg	
Clostridium perfringens epsilon toxin	100 mg	
Shigatoxin	100 mg	
Staphylococcal enterotoxins	5 mg	
T-2 toxin	1000 mg	

Section 9. 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; the NIH Guide for Grants and Contracts; other specific NIH instructions pertaining to the proposed project; CDC guidance documents such as "Biosafety in Microbiological and Biomedical Laboratories"; the DHHS and USDA Select Agents and Toxins regulations; OSHA Bloodborne Pathogen Standard; the provisions of the City of Cambridge Ordinance; Massachusetts DPH regulations for management of biological waste; as well as any MIT Policies and Procedures, and local state and federal regulations.

Specifically, I agree to abide by the following requirements:

a. I will not initiate recombinant DNA research subject to the NIH Guidelines or the City of Cambridge Ordinance until that research has been reviewed and approved by the Committee on Assessment of Biohazards.

b. I will not initiate research with biological agents, human cells or tissues, or select agents and toxins until that research has been reviewed and approved by the Committee on the Assessment of Biohazards.

c. I will make available to the laboratory personnel copies of the approved protocols that describe the potential biohazards and the precautions to be taken.

d. I am familiar with and will use appropriate biosafety level laboratory practices and procedures in this research.

e. I certify that laboratory personnel have appropriate technical expertise and use appropriate biosafety level laboratory practices and procedures in this research.

f. I will ensure that laboratory personnel know the procedures for dealing with accidents and know the appropriate waste management procedures.

g. I will comply with all shipping requirements for biohazardous materials.

h. I will ensure that all laboratory personnel working on this registration are listed with the CAB and will update this list as needed.

i. I will supervise personnel, and correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research.

Nov 1, 2006

Principal Investigator **Date**

MIT Biosafety Officer **Date**

If this project involves the use of human tissue or human subjects, you must contact the COUHES committee at 3-6787.