General Laboratory Manual

Food Safety Laboratory and Milk Quality Improvement Program Laboratory

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(A document under development – your comments and suggestions are requested.)

Welcome to the Food Safety Laboratory, the "Boor" Laboratory and the "Wiedmann" Laboratory. Our overall function is to conduct basic and applied research on the microorganisms responsible for foodborne diseases and food spoilage. Results from these research programs are translated into practical applications through close interactions with extension programs in the Department of Food Science and in other departments. Our laboratories are committed to conduct research at the highest level and to allow undergraduate and graduate students to experience the excitement of research.

This manual is intended to give an outline of the working environment, expectations, and duties for laboratory members. This is a document in development and your input on how to make this better and more informative is appreciated.

For further information about lab procedures, protocols etc., please also visit our WIKI page at: https://confluence.cornell.edu/display/FOODSAFETY/Cornell+Food+Safety+and+MQIP+Lab+Protocols.

Laboratory Mission Statement.

The mission of our laboratories is to conduct high quality research related to microbial food safety, foodborne diseases, and microbial food quality and to support the development of lab members into competent, respected scientific professionals. The laboratory and all its members agree to respect colleagues and to work towards creating a supportive, non-competitive environment. A primary goal of the laboratory is to provide an environment in which students and staff become familiar with all aspects of conducting research at a major research university.

Expectations of Graduate Students.

Graduate degrees at Cornell University are research degrees. The basic requirements for obtaining these degrees are laid out in publications by the Cornell Graduate School and the Field of Food Science. While Kathryn's and Martin's roles as advisors are to help guide you through the process of obtaining a degree, it is every graduate student's individual responsibility to be familiar with the rules and requirements laid out by the Graduate School and the Department.

Time to degree completion:

Outside the rules of the Graduate School, there is no specific upper time limit to finishing a MS or PhD degree. The time necessary depends on research productivity and on completion of the

class work required by one's thesis committee. The expectation is that graduate students will complete a significant amount of independent research that will be presented in a thesis. The speed at which this process is completed is highly dependent on a student's productivity. Obviously, an early start on conducting research is more likely to lead to the completion of a degree within a shorter time frame. Students are encouraged to begin conducting experiments soon after joining the lab. Don't wait – the sooner you get your feet wet the better!

Hours:

Students should recognize that graduate school is rarely a Monday to Friday, 9 - 5 job. Successful completion of experiments often requires long, unpredictable, and eccentric hours. The advantage of being a graduate student, however, is the great deal of flexibility in choosing your hours. However, working hours dissimilar to those of your fellow lab members prevents you from getting to know people and from getting the help you need. In addition, working fewer hours than is generally deemed appropriate can easily become a source of conflict.

All lab members should individually discuss work hours with their supervisors (Martin and Kathryn for postdocs, Grad students, and staff) to establish some regular work hours when they can be expected to be at work. If you will not be in or will be in late, you need to make sure that you let Martin or Kathryn (or your specific supervisor) know through an e-mail or through a phone call.

Vacation:

As a general rule "fall break" and "spring break" are breaks for undergraduate students, but not for faculty, staff, and graduate students (i.e., graduate students are generally expected to work in the laboratory during fall and spring breaks).

The official University policy on vacation for Graduate students is that "a graduate student on an assistantship who needs time away from his or her duties should confer with the special committee chair and faculty or staff member responsible for oversight(s) to establish a schedule for making up the time". Most reasonable people would say that 1-2 weeks/year in addition to University holidays constitutes a reasonable vacation time for grad students. All students need to let their advisors know ahead of time when they plan to be away. Employees need to obtain prior permission of their supervisor to take vacation.

Writing obligations:

In addition to writing a thesis, graduate students are also expected to contribute to the publication of papers in peer-reviewed journals. In general, MS students conduct sufficient research to publish one (or sometimes two) papers and PhD students 2-3 papers. Obviously, the more productive the student, the more papers published. A student's graduation requirements are not considered complete until all papers to be published from his/her thesis have been written.

Scientific meeting attendance:

Attendance at scientific meetings is an important part of your graduate education. Generally, this means presenting your research either orally or as a poster presentation. Meetings that you should consider attending include the Institute of Food Technologists (IFT), the American Society for Microbiology (ASM), Gordon Conferences, the Keystone Conference, the Annual Meeting of the International Association of Food Protection (IAFP), the Annual Meeting of the

American Dairy Science Association (ADSA). These meetings are great places to network with other students and professionals in our field, learn about employment opportunities, and, most importantly, to broaden your scientific background and understanding. Laboratory funds may (depending on our budget…) be available to support travel to scientific meetings for students who are presenting their research. Additionally, the Grad School offers a conference grant program to which graduate students wishing to attend a conference should apply. Please note that the application is **due on the first day of the month prior to the month the conference begins**. Students are encouraged to suggest meetings that they would like to attend. Every student is strongly encouraged to attend at least one meeting before she/he graduates, but students generally do not attend more than one meeting/year.

The scientific literature:

The receipt of a M.S. or a Ph.D. degree from Cornell University implies a certain level of expertise in your chosen area of research. An important component of conducting good research is remaining up-to-date on the current scientific literature. For students in our laboratories this includes the areas of food microbiology, molecular microbiology, infectious diseases, and general microbiology, although additional areas may be relevant to a particular project. Graduate students are expected to closely follow the pertinent scientific literature. This means regularly reviewing the tables of contents and reading pertinent articles from primary scientific journals (e.g., Applied and Environmental Microbiology, Journal of Bacteriology, Molecular Microbiology, Infection and Immunity, Science, Nature or PNAS). In addition, key-word literature searches should be conducted on a regular basis against databases such as Biosis, Medline, PubMed Central and others.

Finally, each graduate student in the lab will be assigned one scientific journal to monitor for articles of general interest to the lab. At the end of each weekly lab meeting, each student should report any articles of interest to the lab which have been published in this journal. It is generally sufficient to bring a copy of the abstract and summarize the contents in 2-3 sentences. In addition, the article reference should be e-mailed to all lab members. The goal of this is to assure that everyone in the lab is aware of the latest scientific work relating to laboratory projects.

General Laboratory Safety.

All laboratory members are responsible for familiarizing themselves with the "Safety Manuals and the General Safety, Handling, and Disposal Procedures" SOP prior to beginning work in the lab. Additionally, all lab members are required to complete a Cornell Environmental Health & Safety session entitled "Chemical Safety for Laboratory Workers" and complete a lab orientation tour with Maureen and Erika. Lab members are expected to maintain a clean and neat bench-cluttered lab benches increase the risk of lab accidents and contamination problems.

If you are at any time unsure about proper safety procedures in the laboratory ask an experienced lab member for help and clarification. If no one is in the laboratory, you can call Martin (227-5903; cell phone) **at any time** for help.

In case of an accident or injury while you are in the lab, inform Sherry Roof or Nicole Martin **IMMEDIATELY.** An accident must be reported through the university's HR web page.

Should you need emergency services, they can be contacted by dialing "911" from any lab phone or 607-255-1111 from a cell phone. The accident/exposure procedure is posted above every eye wash station in the lab.

Bio-Safety.

The lab follows the "Cornell University Biological Safety Levels 1 and 2 Written Program." A copy of this is kept with the Chemical Hygiene Plan, and MSDS sheets on the shelf in 352.

The FSL operates at a biosafety level of 2 (BSL-2), which allows work with a variety of pathogens that can cause disease in humans (e.g., *Salmonella* Typhimurium, *Listeria monocytogenes*). Eating and drinking in the laboratory is not permitted. Waste contaminated with infectious substances needs to be placed in designated waste bins. Reusable materials contaminated with infectious substances need to be placed in the proper plastic buckets so people responsible for washing glassware can handle these materials appropriately. Refer to the safety manual, or ask Maureen, Sherry or other staff for help and explanations as to where and how to dispose of infectious substances and other hazardous waste.

There are several different kinds of waste containers for BSL-1 and BSL-2 waste: large boxes, benchtop cylinders, pipet buckets, and sharps containers, both large and small. There are also basins beside the sinks which are specific for BSL-1 waste or glassware to be washed, **but only one which is for BSL-2 liquid waste**. BSL-2 containers can be recognized by their red biohazard bags or red plastic. All waste containers should be plainly labeled, but the quick way to tell is, if it's red, it's BSL-2.

Chemical Safety.

The lab follows the "Cornell University Chemical Hygiene Plan" and the "Hazardous Waste Manual" which are located on the shelf in 352. This is a "living" document, changes may be made by EH&S from time to time. When you are looking for information regarding chemical safety, please access the most current copy which is located on the EH&S web-site at: **sp.ehs.cornell.edu/lab-research-safety/laboratory-safety-manual/Pages/default.aspx**

Experiments in the laboratory sometimes involve the use of chemicals and reagents which might represent a possible hazard to human health. A hardcopy of the Material Safety Data Sheets (MSDS) for hazardous reagents used in the lab are located in a black binder on the shelf in 352.

You can also find the MSDS through ChemWatch at: **https://sp.ehs.cornell.edu/lab-research-safety/research-safety/msds/Pages/default.aspx**

Laboratory Notebooks.

You will be provided with a laboratory notebook in which to record the methods, results and discussion of your experiments and into which to attach any relevant pictures, printouts, procedures, etc. Laboratory notebooks are the property of the FSL and are kept on file after you leave the lab.

All laboratory personnel conducting research are expected to read Chapter 5, "Laboratory Notebooks", in *At the Bench: a Laboratory Navigator* by Kathy Barker, paying particular attention to the "Content" section on p. 92. The level of detail required in keeping a notebook is such that (i) you could go back to it (even months later – as is very common) and understand what you did and (ii) another scientist could pick it up and easily repeat the experiment. In addition, it is not uncommon for an experiment to "fail" for unknown reasons. However, at some point in the future you might obtain additional information that enables you to re-interpret that "failed" experiment. Yes, it takes a great deal of time to keep a good, detailed laboratory notebook, but it is time well spent. The resulting record is essential in writing a thesis and papers and represents a permanent record of research conducted in the lab.

Laboratory notebooks will be reviewed weekly by Kathryn, Martin or your direct supervisor as an aid for reviewing lab projects.

Key points regarding lab notebooks

- Record everything as soon as possible, including calculations!!
- Use only pen
- Do not erase, white-out or otherwise obliterate mistakes, simply cross out with a single line
- Notebooks are the property of the FSL and are kept on file after you leave

Laboratory Meetings.

Our laboratories hold weekly joint laboratory meetings, the purpose of which is to discuss recent research topics and ongoing research projects. Attendance is expected of technicians, graduate students and postdocs and is encouraged for undergraduates. Day and time for lab meetings is set to optimize broad participation by lab members. The general format for these meetings is: (i) 20-30 min in-depth research update by one lab member, (ii) brief status reports of ongoing research projects, and (iii) miscellaneous other lab-related topics.

For the in-depth research update/presentations, each week a graduate student will make a ~20 – 30 minute presentation on their own research results and/or research plans. A schedule of lab talks will be sent out at the beginning of each semester. You may switch your time with another student; however, don't forget to inform the person setting up the lab talks. A research presentation should consist of a brief introduction on the background and goals of your project and then focus on data that you have acquired. Technical difficulties should be brought up for discussion. Our goal is to provide a safe, comfortable environment in which graduate students can learn to present scientific data and in which all lab members can participate in a meaningful scientific discussion. The presentations are informal.

During the second part of the meeting, each lab member will have an opportunity to make general lab announcements and discuss other related topics.

Food Safety/Food Microbiology Journal Club.

All Graduate students in both Martin's and Kathryn's lab are expected to attend all journal clubs and to present one paper per semester.

The book *At the Bench: a Laboratory Navigator* by Kathy Barker has an excellent discussion of how to choose topics for and give journal presentations (pp. 116-118). New lab members will have the chance to observe several presentations by more experienced members prior to their own first presentation.

The Financial Reality of a Research Laboratory.

Research in the FSL is funded through a variety of sources, including federal funds (i.e., taxpayers' money), funds from private companies and funds from different organizations (e.g., Dairy Management Inc., which is funded through contributions from US dairy farmers). Most of these are competitive funds, meaning that we must write proposals that are judged against proposals from other laboratories. Our ability to secure funding for research projects depends entirely on our record of sound research (generally measured as publications in peer-reviewed journals) and on a good reputation for the laboratory and its members. Without obtaining these funds, we cannot maintain a research program, pay salaries for technicians and undergraduates, support graduate students, and buy necessary supplies and equipment.

Strain Collections and Databases.

Many of the projects in the FSL are dependent on our large collection of bacterial isolates, as well as on the generation of new recombinant bacterial strains and plasmids. Therefore, our ability to maintain viable, pure cultures in long term storage (usually frozen at -80°C) and to keep track of the identity and location (via our strain database) of these cultures is crucial. The importance of maintaining strict aseptic technique in the preparation of frozen stock cultures and of accurately recording strain information in the database cannot be overemphasized. Experienced lab members are responsible for instructing and overseeing new members in these activities.

New microbial strains and isolates are named with a system consisting of the prefix "FSL", an identifier specific for each person in the lab (e.g., W1) and consecutive numbers. FSL number assignments are handled by Maureen. A sheet describing the rules for naming new strains and isolates is attached. Each lab member is responsible for properly naming, preparing frozen stock cultures, and recording all strains generated or collected during their project in our WWW-based Food Microbe Tracker database (www.foodmicrobetracker.net); please make sure you get a password for Food Microbe Tracker so that you can enter your strains. All isolates and new strains need to be frozen in a timely fashion (i.e., within 1-2 weeks after construction or isolation). Before students will be able to graduate, we will check the Food Microbe Tracker database against your lab book to make sure all your isolates and strains are entered in the database.

Newly constructed plasmids are named using the creator's initials and a consecutive number (e.g., pMW1, for the first plasmid constructed by Martin). Aliquots of purified plasmids and stock cultures of host strains containing the plasmids are stored frozen in the -80°C freezer. Detailed plasmid descriptions and storage location are entered into the plasmid database.

An additional important database is that for our primer sequences. Primers are labeled to reflect the targeted gene (e.g., actA-F) and are given the net ID of the designer plus a consecutive number (see protocol for naming and ordering primers on p. 10). They are then recorded in the primer database (Nancy Carey presently enters these as they are ordered).

Ordering.

One or two staff members in the lab are designated to be responsible for requesting stock laboratory supplies and reagents on a regular basis (currently Jordan Skeens and Maureen Gunderson). However, ALL lab members are responsible for requesting items to be ordered when they are running low and BEFORE they run out, so please anticipate your needs for materials, chemicals, and reagents to avoid rush orders. Items in need of ordering should be recorded on the clipboard located on the benchtop in room 358 Stocking Hall and also sent to Nancy Carey (nrs13), who is in charge of ordering, in a mini Excel spreadsheet as below. Fill in all the information you have available and be sure to include your NAME. (Note: More rows can be added, but please do not delete any columns.)

Date of Req.			Catalog No.	**Company**		Item or Service	Size	Quantity				Comments	Name

Computers.

Our laboratories maintain a number of computers for use by lab members. Use of computers for laboratory related activities (manuscript preparation, data analyses, etc.) have priority. Personal use of laboratory computers (WWW cruising, game playing, and personal e-mail) should be limited to off-hours.

All lab-related electronic documents should be saved to the food science file server. Photos, videos, and other non-work related documents are not allowed on the file server. Instructions on how to connect to the file server can be found posted around the lab and in protocol 5.13-Mapping_Printer_Server_RemoteAccess.

Telephone usage.

The laboratory telephone is set-up for campus and local calls. Long distance calls require an access code or a credit card. The lab receives a monthly statement of all telephone calls placed from the lab. Please use your personal cell phone, a credit card, or a phone card for personal long distance calls. Department policy prohibits personal long distance phone calls to be charged to our accounts. Please see Martin or Kathryn if you have any questions or concerns about this.

Suggested readings.

The manual *At the Bench: a Laboratory Navigator* by Kathy Barker is an extremely useful "guide" to life in a research lab, especially for new graduate students. The first section (General Lab Organization and Procedures) covers topics related to getting oriented in the lab; the second section (Laboratory Setup and Procedures) discusses the rationale and organization involved in setting up experiments, recording results, and presenting data; and the third (Getting Started and Staying Organized) presents details on various common lab protocols. All lab members conducting research are expected to read Chapter 5 "Laboratory Notebooks".

Recommended reading:

Basic Survival Rules, pp. 15-17

For nonnative English speakers, p. 104

Journal Clubs, pp. 116-118

Additional Resources.

There are a variety of additional resources and books, which can provide important background information on your experiments, experimental procedures, etc. Please consult them regularly to gain a thorough understanding of the experimental procedures you are using. The following is a list of selected resources. Please let Martin know if you know of other resources which you think should be listed here:

Bergey's Manual of Systematic Bacteriology. This is the "bible" of bacteriology. After a couple weeks in the lab you should have looked at this book at least once. It tells you a great deal about the characteristics of the different bacterial species and genera. Microbiologists refer to it lovingly as "Bergey's". This is a 4 volume set which is located in room 352.

Molecular Cloning: A Laboratory Manual (by Sambrook, Fritsch, and Maniatis). This is the "bible" of molecular biology. This is the other book you should have consulted at least once if you have been in the lab for a couple of weeks. This manual not only provides detailed protocols for many molecular biology techniques, but also gives information as to why we do certain things the way we do them. If a protocol you use is very different from what's outlined here, ask **Martin** or **Kathryn** for a reason (always remember that protocols you get from someone who got them from someone, etc., might not always be correct). Microbiologists refer to it lovingly as "Maniatis". This is a 3 volume set which is located in room 352. The most recent edition, which does not have Maniatis' name on it, is also located there.

Current Protocols in Molecular Biology (by Ausubel et al.). Similar to Maniatis, this is a protocol book that provides detailed protocols for various molecular biology experiments and procedures. This is a 4 volume work, which receives regular updates. Please consult this book for any new molecular biology procedures you will be using in your experiments or to learn more about techniques you are using. More up to date copies of Current Protocols can be found in Mann Library.

Listeria, Listeriosis, and Food Safety (by E. T. Ryser and E. H. Marth). This book will tell you everything you ever wanted to know about Listeria. A good resource if you are working with Listeria and feel you need some more information about a specific aspect of this organism. Located in Martin's office.

Genetic Analysis of Pathogenic Bacteria: A Laboratory Manual (by S. R. Maloy, V. J. Stewart, and R. K. Taylor). This lab manual provides some outstanding information on different techniques used in the genetic analysis of bacterial pathogens. It is not as comprehensive as Maniatis, but generally provides more details and more background information. Start reading it from the beginning if you have some spare time or boring visitors coming for the weekend.

Microbial Genetics (by S. Maloy, J. E. Cronan, and D. Freifelder). This is a textbook and a good resource to look up things. Located in Martin's office.

The New England Biolabs Catalogue. This catalogue provides valuable information on use of different restriction enzymes, buffers, etc. Useful resources are located in the beginning and the end of this catalogue. If you use molecular biology techniques, make sure you flip through this at least a couple times to know what type of information you can find in there. There are multiple copies of this catalogue at various locations in the labs. Additionally, www.neb.com is a very useful web resource, and there are helpful apps to download, such as double digestion tables.

Emergencies.

Campus emergency personnel can be reached by dialing **911.**

Kathryn (227-5832; cell phone) or Martin (227-5903; cell phone) can (and should) also be called **at any time (day or night!)** for help.

FSL ID Number Assignment.

FSL isolate designations are assigned to staff & students freezing strains for permanent addition to the FSL isolate collection, which is maintained at –80°C. The FSL isolate numbers are assigned by either the PI or person in charge of the –80°C freezer organization (currently Maureen Gunderson). Designations consist of the first letter of an individual's last name and a pre-determined number. There is a list of current FSL isolate designations posted on the -80C freezers that is periodically updated.

Example: Joe Schmoe's FSL isolate designation will be S7. The first isolate that Joe assigns a number to will be assigned FSL S7-0001. Joe's isolates will all have S7 designations regardless

of the project they are associated with until Joe reaches FSL S7-9999. At that time, he will receive a new designation due to database constraints.

In general, whenever a new isolate or strain enters the lab or is created within the lab, it should be given an FSL ID number and frozen as a glycerol stock. (Before preparing glycerol stocks, refer to the FSL Isolate Designation, ID Assignment & Glycerol Stock Preparation Protocol on the Food Safety Wiki). For clarification purposes, the following is a list of specific examples of isolates or strains that need to be given FSL numbers:

- An isolate is received from an external source.
- A new isolate is recovered from a sample or enrichment.
- *E. col*i containing a plasmid constructed with an insert or deletion or reporter gene.
- *Listeria* containing a plasmid constructed with an insert or deletion or reporter gene.
- *Listeria* mutants.

Do not re-assign internal FSL isolates with a new FSL number once you begin working with it (i.e. 10403S is FSL X1-001…*forever*).

Naming of Synthesized DNA Oligonucleotides (including PCR Primers).

All synthesized oligonucleotides need to be named according to the following system. Assign every sequence you design with your net ID and a sequential number. A brief description, as usual, may follow. The primer name should <u>not</u> have spaces, periods, commas, or special characters. Underscores or dashes may be used. For example:

AG67-16-cdtB-F
[*Net ID*] [dash] [*sequential #*] [dash] [*brief description*]

This primer was designed by Ahmed Gaballa (ag67), was the sixteenth sequence he designed, and is the forward primer for a *cdtB* PCR.

1) This applies to all regular and Real Time PCR primers ordered from IDT.

2) This also applies to all TaqMan probes ordered from Applied Biosystems or MegaBases.

3) If a primer is redesigned (for example, the PCR didn't work so you're trying a new primer), the new primer *must* receive a new number.

Ordering Primers.

To order primers, choose the next consecutive location(s) on the freezer sheets in room 352C and fill in all data. Send your order request in a mini Excel spreadsheet to Nancy (nrs13) with the following information for each primer:

Order Request Date	Freezer Location	Primer Name	Sequence