**Reinke Lab Information and Policies**

**Winter 2023**

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Basic Lab Information

PhoneLab phone: 416-946-0920

Aaron’s office phone: 416-946-0889

## Shipping address

University of Toronto
661 University Ave

MaRS Centre, West Tower, 16th fl.
Toronto, ON M5G 1M1 Canada

## Fedex #

## Personnel Contact info

Please add your information to the list:

# Lab Communications

## Lab website

[www.reinkelab.org](http://www.reinkelab.org)

## Lab email

## Lab Instagram

<https://www.instagram.com/sporeposse/>

## Lab Slack

Everyone in the lab should be signed up for Slack, including all undergraduates, so that we can communicate in case of an emergency. There are also several channels (deliveries, papers, science seminars) that all full-time people in the lab should join.

## Regular lab zoom link

Request for letter of recommendations, editing fellowships, ect…

Please let Aaron know at least a week ahead of time if you need any documents or editing from him.

# Lab Safety Orientation

All new lab members must complete safety training – both U of T and go over training with Winnie. Safety refresher course has to be taken every year. Safety information is in our shared lab folder.

# Lab files and IT

## pCloud

We are currently sharing everything through pCloud.  I have shared with everybody the general lab folder, that has safety, lab meeting, ordering ect.  I also created a project folder for everybody and shared that individually. I would like everybody to store all of their electronic data in their project folder and locally on their own computers, so that way all data is always saved in the cloud and locally.  The one exception is for large sequencing and imaging files. There are separate data folders for these, and we can save them on the PC in the dry lab in these pcloud folders. We will back up the microscope images every 6 months onto external hard drives.

To use pcloud, sign up for a free account.  Then down pcloud drive.  This will give you a virtual drive to see shared folders.  You will then need to sync your project folder so that those files are physically saved on your computer.  You don't need to sync the general lab folder or data folders unless you want to.

If you don’t use pcloud for saving data, you must use some other cloud service to save data. No electronic data should be stored only on one’s laptop.

## Reserving autoclaves and meeting rooms

## Computer and software:

If you need computers or software for anything, talk to Aaron and we can purchase these things.

Biorender info:

## For cluster computing we use Compute Canada

<https://ccdb.computecanada.ca/account_application>

Use the server "Graham"

<https://docs.computecanada.ca/wiki/Graham>

<https://docs.computecanada.ca/wiki/Available_software>

Talk to Aaron if you have any questions on how to use. Lina has also created a user guide for the cluster. P:\RL\_General\Protocols\Compute Canada

## Printer

We have access to printer that is connected to lab computer. It might be possible to connect this to your own computer. I believe we also have access to a departmental printer on the 15th. Below is how we use to set up the printer on the 16th, which is probably similar for using the one on the 15th.:

We have access to the Ricoh on the 16th floor (IP address 142.1.243.30 )

The driver for Windows is at <http://support.ricoh.com/bb/html/dr_ut_e/rc3/model/mp6503/mp6503.htm>

# Lab organization

## Meetings

Lab meeting and journal club schedules:

Please upload presentations you generate for these meetings to the presentations lab folder.

## Lab Calendars

We have three calendars:

Events- For lab events and to indicate days out of town.

Microscopes-For scheduling microscope use

See microscope use policy-

Equipment-For scheduling all other equipment

Please use these calendars to help your fellow lab members to schedule their time and experiments. You can ask Winnie or Aaron to add you.

Lab Notebooks

You are expected to keep a complete and accurate record of your work. This can be done in a number of ways, according to your personal style. You can keep your notebook either physically or electronically. Be sure to record enough detail so that you can write the methods section of a paper without having to go back and do everything over again. Keep meticulous experimental notes and be sure your notebook is up to date.

# Ordering

Winnie does most of the order and Aaron also has ordering access. If you need something ordered, enter the request in Quartzy. Winnie can add you if don’t have access.  If you don’t know the vendor/catalogue number, hit “skip lookup” and type “None” for the vendor and “don’t know” for the catalogue number. If commonly used consumables are low, please add to the list as soon as you notice this. If you are ordering a new item that we haven’t order before, get prior approval from Aaron.

## Ordering worm strains

Aaron can order worms for you through the CGC. He might also know local labs that are likely to have the desired strain.

##

## Receiving

Bring attention to the person who ordered the item. This can be done through slack and should also be marked as received in Quartzy. Perishable items need put away immediately upon receiving. Write down the date of receipt and your initials on the item.

## Media requests

We will try to maintain common lab stocks of M9 and seeded and unseeded NGM plates, and 10XOP50. Orders are generated on a “week of” basis but your numbers must be requested by the start of ***Tuesday prior*** to this. This will allow our undergraduates to pour the necessary number of plates and give them time to seed what will be needed.

If you need both seeded and unseeded, please fill in your name slot with the appropriate numbers. For example, if you need 20 seeded and 40 unseeded 6cm plates, please fill 20xS, 40xU. There are two tables for filling out your plate needs for 6cm and 10cm plates.

You don’t have to take all of the plates you ask for, but you should refrain from taking more than you’ve requested.

There is a rotation for helping with Lab seeding. All fulltime staff doing *C. elegans* work are expected to spend a week every month or so helping with seeding plates.

# Lab Equipment Information

## Equipment

Do not use any equipment without being trained on it first. Only the person in charge of training on that instrument may train you. Do not use other lab’s equipment without first talking to Aaron, or someone in that lab. Other labs may use our equipment but only after being trained on it by someone in our lab’s person in charge of training for that piece of equipment. Here is link to shared equipment available in MARS:

There are also several core facilities that we use if we need special equipment. If any equipment breaks, let Aaron know as soon as possible.

**Equipment passwords**

## Lab space and storage in incubators, cold room, and -80

Lab space:

1. Ideally, all full time members are ideally assigned their own bench. If this is not possible, we will come up with a plan to be able to efficiently share space.
2. Try to keep your lab benches as clean and uncluttered as possible so that it is easy for others to use.
3. Be sure to put the days you are gone on the lab calendar so that everyone can see where space is free.

**Incubators**

We have large 16, 21, and 25 degree incubators. Do not change these without talking to Aaron. We also have a small under bench variable temperature incubator, and a 37 degree incubator. You can use what ever space is free in these incubators, but do not move other people’s boxes without talking to them. Do not leave plates around for more than 1-2 months without them being parafilmed. Quickly inspect plates for mites before throwing them out. If you notice mites, let everyone know so that everyone can inspect all of their plates.

<https://www.youtube.com/watch?v=3pn2BdyB3KY>

**-80 Freezer**

We have areas reserved for lab reagents and then assigned spaces for each member. Talk to Winnie if you need more space. Check temperature before opening and if warmer than -75 don’t open. Don’t keep open for more than ~30 seconds at a time.

**-20 Freezer and refrigerator**

We have areas reserved for lab reagents and then assigned spaces for each member. Check temperature before opening and if warmer than -20 don’t open. Don’t keep open for more than ~30 seconds at a time

Please keep storage of excess items, such as tubes, plates, and microscope slides to a minimum. Go through items at least every 6 months and discard items that aren’t needed and to consolidate items to take up less space.

## Room 1665

The front part of the room has equipment that requires some amount of light to use including our clean cabinet, injection scope, and incubators. The back part of the room contains our fluorescent scopes as well as scopes from the Houry and Frappier labs. This room is designed so that both halves can be used at the same time. If no one is using the microscopes in the back the ceiling lights may be used. When people are using the microscopes, use the light in our clean cabinet. We always have the right to have this light on, and it is up to other labs to insulate their scopes if they need a darker environment.

The clean cabinet in this room should be turned on 10 minutes before using. When done please turn off, unless someone is planning on using it next.

If you are likely the last person to leave for the day, please make sure that the microscopes and clean cabinet are turned off. If there are still plates in the clean cabinet, leave on, but send out a message to let everyone know.

## Equipment temperature monitoring

Ree’s monitoring has been set up for the lab -80 freezer, the -20 freezer, and the 21 degree incubator. Login here:

Currently the phone list is set up so that if one of the equipment goes out of the preferred temperature limits, the lab phone gets called first, followed by the office phone, then Winnie’s cellphone, then Aaron’s cellphone if none of the other calls get picked up.

If there is a disruption in temperature that is noticed either through this monitoring, or you see the temperature being wrong in the lab, first try to get in touch with Aaron and then email the rest of the lab. In case of an emergency with the -80 we will decide if boxes need to moved. If boxes need to be moved, first make sure there is dry ice available and space in the back up -80 (these are located on the south end of floor, next to the south entrance into the labs). Highest priority is shelf 2 (this is where all of the worm strains and spore preps are located.) Try to minimize the amount of time the -80 is open.

# Lab Protocols and Etiquette

## NGM plates

Everyone in the lab shares from a common pool of seeded 6cm and 10cm plates that are used for various experiments and upkeep of strains. In order to prevent sudden shortages, it is important to use the plate sign-up sheet on the 20C incubator.

## Flame etiquette

Don’t leave Bunsen burners on unattended. Keep the wicks on the alcohol lamps short enough that the flame they give is not at a dangerous level.

## Waste

We have several kinds of lab waste:

Chemical waste goes in green buckets. This is weigh boats, paper towels, tips, and serological pipettes that haven’t touched any biological organism. When full, dump into big blue barrels in waste hallway.

Biological waste goes in yellow bin with liners. This will be picked up by staff and disposed of.

Paper can go in black trash can. Cardboard goes in blue buckets outside lab on the west side of the floor.

Liquid waste such as PFA and trizol is to be collected at the bench and then poured into larger marked containers in fume hood. Once these containers are full they can be picked up.

Everyone should have a beaker for *E. coli* and worm waste. After this waste has been bleached it can be dumped down the sink. Please keep sink clean of agar and agarose. Do not use excessive amounts of bleach. If there is a noticeable bleach smell, cover the beakers.

## Working with common reagents

### Nematode Reagents

Take 10x op50 aliquots from bottles using serological pipettes. Make sure it is carefully resuspended before using. If you will be using more than a few mls, you can take your own 50ml conical. Only use 10x op50 that is less than a month old.

Take your own bottles of M9 for your own use. Check seeded NGM plates carefully for contamination before using. If more than a few plates in a box are contaminated, indicate on the box and let Winnie or Aaron know. Wipe down empty worm boxes with bleach before putting back. Put empty pipette boxes and tube jars in the area marked for them.

### Streakout microbes and thawing worm strains

Place all microbes and worm boxes on -80 while finding the tube you want. Keep on dry ice while streaking out microbes.

Statistical thinking for C. elegans experiments:

http://www.wormbook.org/chapters/www\_statisticalanalysis/statisticalanalysis.html

Reproducibility and working with worms:

It is important to think about sources of variability when working with *C. elegans*. Read the following paper for a good overview:

elegans biology. Biochemical Society Transactions 47(3):BST20190001.

Pho KB, MacNeil LT. (2019) Biology is the root of variability: cautionary tales in Caenorhabditis elegans biology. Biochemical Society Transactions 47(3):BST20190001.

Known sources of variability are the following:

Age of *E. coli*- don’t use seeded plates or 10x op50 stocks more than 1 month old.

Temperature. Keep all experiments in incubators as much as possible and make sure plates and liquids are at room temp before adding to worms.

Starvation – All worms should be well fed for at least 3-5 generations before using for experiments. The PO generation is the most important and if this generation experiences starvation then be skeptical of F1 results.

Bleaching. Don’t use bleach solution that is more than 6 months old. Keep this bleach at 4c and wrapped in foil. Always monitor F1 worms. If there is a high proportion of unhatched embryos or sickly looking L1s, these animals are not likely to be useable.

Spores

We try to keep common spore stocks of *N. parisii* (ERTm1) to be used for most experiments. These need to be taken out of the -80 very quickly to avoid them being thawed. If you haven’t taken them out before, use a box of dry ice. If using a large number of spores check with Winnie or Aaron first to make sure we have enough.

For winter/summer 2023 Spore preps for *N. parisii* will be in teams:

Winnie and Ed

Hala and Angcy,

### Molecular Reagents

PCR reagents are made in common stock aliquots. When working with common reagents such as dNTPs and PCR buffers, please take a single aliquot and store in your own personal reagent box. This will avoid confusion and potential shared contamination with other lab members.

When working with enzymes (Restriction enzymes, polymerases, ligase, etc) keep these at -20 until you are ready to add them to your reactions/master mix. Use the cold blocks to transport these from the -20 to you bench and return these immediately after adding them to your tubes. Always use filtered tips when working with these reagents and centrifuge/spin these down before working with them to reduce wastage.

## Reagent submission for lab collections

All DNA constructs, *C. elegans* strains and microbial strains should be submitted to the lab collection. This is done using our elab inventory account.

*C. elegans* strains that we generate in the lab need to entered following established nomenclature. Check with Aaron if you haven’t done this before. Use the spread sheet below to take both lab and allele numbers:

 DNA strains can be named by using your three initials and numbering sequentially.

All submitted strains should be placed in the main lab collection of strains. Freeze down 3 copies of each worm strain. 2 copies will be kept in -80 and 1 copy will be placed in liquid nitrogen. If you thaw the last copy at -80, freeze down 2 copies to replace.

Freeze down at least one copy in cornmeal.

https://pubmed.ncbi.nlm.nih.gov/34712912/

Lab Jobs for Winter/summer 2023

 Training on microscopes – Hala/Ed

 Training on injection scope – Winnie

 Training on PCR machine/shaker and CL2 safety - Yin

-80 organization, -20 and refrigerator organization - Angcy

**Resources for writing, making figures, preparing posters and giving talks:**