Tirosh Lab Book

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Chapter 1

Homepage

1.1 Links

1.1.1 Useful

- Dropbox home
- Lab publications
- Lab website
- Experimental protocols

1.1.2 Fun

- Lab Twitter
- Birthdays
- Lab members
- Lab trip photos

1.2 Adding to the book

Adding to the lab book is easy. The only requirement is that you make a Github account, which shouldn't take more than a couple of minutes to do.

Note that since the book is re-built with each edit made, **you may have to wait a few minutes before your changes become visible**.

1.2.1 Option 1

1. Go to the website chapter that you would like to edit.
    - E.g. Chapter 3 Lab members and contact details
2. Click on the edit icon at the top of the website page.
3. Make your edits and submit them (green button at the bottom of the page).

1.2.2 Option 2

Note: this will only work for those whose Github users have been added to the book repository.

1. Go to your local clone of the repository.
    - to clone the repository: `git clone https://github.com/tiroshlab/lab-book.git`
2. Make and save the relevant changes to the .Rmd file in question.
    - There is one .Rmd file per chapter.
3. Submit the edit. In the terminal type:
    - `git commit -am <myShortMessage>`
    - `git pull origin master`
    - `git push origin master`

1.3 Book formats

The default book is a website. You can also choose to download the book as PDF or ebook. To do this, click the download icon at the top of the website page.

Chapter 2

General research directions of the lab

We are a cancer systems biology lab, focused on intra-tumor heterogeneity (ITH). Our main goal is to understand ITH, the mechanisms that generate and maintain it, and the functional and clinical implications.

2.1 ITH mechanisms

We consider three main types of mechanisms: 1. Genetic heterogeneity (genetic subclones). 2. Microenvironmental influences (e.g. oxygen/nutrient availability and cell-cell interactions) 3. Intrinsic cellular plasticity (cells dynamically transitioning between states).

2.2 ITH functional/clinical implications

We are interested in all implications of ITH but focus primarily on the questions of drug resistance, cancer stem cells and metastasis/invasion, all of which have highly significant clinical implications.

2.3 Approach

We use a systems biology approach, which means that we combine experimental and computational approaches to address these questions from a global perspective, typically through single cell RNA-seq or other high-throughput profiling

approaches, followed by extensive computational analyses, and experimental validations.

2.4 Experimentally

We emphasize the ability to work directly with patient samples and to that end we work closely with clinicians. In addition, we work with "simple" model systems (cancer cell lines), but only after we verify that they recapitulate certain aspects of heterogeneity as seen in tumors. We tend to avoid working with complex models and with experimental systems that are difficult and slow to work with, such as mice models, although this is possible through collaborations with other labs. Our main experimental method is scRNA-seq, but we try to combine it and follow up with complementary approaches (FACS analysis, spatial analyses, time-course experiments, ATAC-seq) which will expand and evolve with time.

2.5 Computationally

We emphasize a biology-centered and data-driven approach, and hence perform hands-on extensive, iterative, and integrative data analyses while avoiding the "blind" use of computational tool. We do not focus on any particular computational methodology but attempt to use the appropriate method for each analysis, while keeping the analysis intuitive and simple.

2.6 Biological contexts

We study primarily glioma and head and neck cancer. These are long-term directions in which we collaborate closely with experimental/clinical groups (Mario Suva from Boston and Sid Puram from St. Louis), striving to deeply understand the underlying biology and ultimately make a clinical impact. We are currently starting also a third focus on neuroendocrine tumors (NETs), in collaboration with Amit Tirosh from Sheba Medical Center (no family relations). In addition, specific projects involve other contexts due to either exceptional diversity (e.g. Carcinosarcomas: tumors that contain both carcinoma and sarcoma cells), or collaborative efforts (e.g. in ovarian and pancreatic cancer).

Chapter 3

Lab members and contact details

1. Itay Tirosh, tirosh.itay@gmail, 054-9453547, Hanasi Harishon 54 apt. 20, Rehovot
2. Alissa Greenwald, aliscohen@gmail.com, 054-5761984, Uri Zvi Grinberg 1A, Rehovot
3. Rony Chanoch, ronychanoch@gmail.com, 050-5982514, Moshe Sharet 3 apt 11, Tel Aviv
4. Mike Tyler, m20tyler@gmail.com, 058-6594177, Derekh Yavne 81 apt. 25, Rehovot
5. Avishay Spitzer, dr.avishayspitzer@gmail.com, 052-8365141, Hameri 18, apt. 3, Givatayim
6. Michael Mints, michael.mints@umu.se, 058-5734784, Sharabi 2, Tel Aviv
7. Julie Laffy, jlaffy7@gmail.com, 058-7075989, Dizengoff 149 apt. 3, Tel Aviv
8. Noam Hadary, noam.hadary@weizmann.ac.il, 052-4323212, Derech Yavne 63, apt. 14, Rehovot
9. Noam Galili, noam.galili@weizmann.ac.il, 054-3173130, Beit Tsuri 11, Tel Aviv

Chapter 4

Specific Projects

Alissa
My two main projects are 1) Modeling ITH in cancer cell lines with a focus on dynamics, differential drug sensitivity, and cancer cell-cancer cell interactions in HNSCC (with Rotem). 2) Spatial transcriptomics of GBM (in collaboration with the Suva Lab).

Rony
My thesis focuses on GBM intra-tumor heterogeneity, for which I have 3 projects in collaboration with Mario Suva's lab at MGH: 1) modeling the GBM states in cancer cell lines and a mouse model of GBM, 2) exploring the GBM microenvironment cell-cell interactions in regards to the cellular states, and 3) targeting the GBM states as a therapeutic approach for GBM.

Mike
My work concerns deconvolution of bulk RNA-seq data to identify signatures of tumour subpopulations or cell states. My current project focuses on distinguishing EMT (or partial EMT) from stromal cells in bulk transcriptomes.

Michael
My work broadly focuses on ITH in head and neck cancer. In collaboration with Sid Puram's lab I analyse scRNASeq data from patients with oropharyngeal, laryngeal and oral cavity cancer in order to find tumour genetic subclones and functional metaprograms in the cancer cells and microenvironment.

Noam H
My work is to do a regulatory follow up to lab discoveries on chromatin organization level using single cell ATAC-seq on two main ITH projects: 1) head and neck cancer 2) GBM.

Julie
My work focuses on understanding expression heterogeneity in gliomas. I analyse primarily scRNA-seq data from the Suva lab to 1) define the subpopulations

of cancer cells that exist in different glioma types, 2) understand the emergence of these subpopulations as a function of cell lineage, genetics and the TME and 3) revisit the distinctions between glioma types.

Noam G
My project focuses on deconvolution of bulk RNA-seq data to identify and distinguish IFN response and MHC class ii genes in cancer cells from immune cells.

Chapter 5

Joining and Leaving protocol

5.1 Joining

When joining the group:

1. Ask Michal Ovadia to pool your userID to this department and connect you to any services you may need to use (at least WEXAC and email will be necessary, and RSA very useful), note, if you have userID in other department, pooling you will erase your old server folder.
2. Ask Michal to add you to the lab dropbox.
3. Order a computer – available options are found at the DIS site Check with Itay before ordering.
4. Natali Yikne is the one that supplies network cable, opens the network connection point near to your desk and makes your registration to the internet - for that, you will have to supply your ETHERNET code (found on the Ethernet outlet) and the computer's MAC address.
5. Ask Itay to get the lab google calendar so you can update and know future plans.
6. If you're a foreigner, go to the FGS for help to set up banking and insurance.
7. Also as a foreigner you can arrange housing through the visiting scientist centre.
8. Provide your bank account to Michal so you can get paid.
9. After registering you will get emails about doing a medical checkup, lab safety introduction and having your photo taken so you can get a Weizmann badge.

5.2 Leaving

When leaving the group:

1. Make sure to return all your computer accessories (docking station, charger, mouse, keyboard etc)
2. Make sure to back up your code, results and data, as your folder will be erased.
3. Talk to Michal to remove you from services.
4. Return your badge to Michal when you leave.
5. Notify Natali whether you possess Weizmann laptop and accessories and who will get that after you.

Chapter 6

Lab Responsibilities

1. Ordering (scientific, food)
    - warehouse orders & office supplies - Alissa + Rony
    - milk & machonkolet
    - coffee & coffee machine supplies
2. Group meetings and journal club organization
    - Julie
    - Mike
3. Lab trip and lab week organization
    - Rony
4. Equipment/reagents maintenance
    - autoclave - preparation of materials (eppendorfs, Pasteur pipettes, H20) and transporting to/from autoclave on 3rd floor
    - waste disposal - bringing chemical and biological waste to the waste disposal closet (and replacing with new bags/containers), emptying vacuum traps in tissue culture and main lab and refilling with bleach - Rony
    - tissue culture maintenance - replacing water pans in the incubators and treating with Aquaguard (biweekly to monthly), replacing water in the water bath and treating with Aquaguard (monthly), autoclaving incubator racks (bi- annually), disinfection in case of a contamination, keeping the tissue culture room stocked with supplies - Alissa
    - aliquoting of common reagents - aliquoting FBS, pen-strep, glutamine, Aquaguard 1&2 on a regular basis for the whole lab and keeping these reagents in stock - Alissa
    - coffee machine maintenance

5. Lab code
    - Julie
    - Avishay
6. Lab organization/setup (esp. including when moving to the new building) - Alissa + Rony
7. Lab safety - Alissa

Chapter 7

Shared Data

7.1 Datasets

Single-cell RNA-seq datasets that are commonly used by the lab are on the server home under `shared/datasets`. Each dataset is a separate .txt file and the files are categorised into subfolders by cancer type (or "Normal").

For more information on the datasets found here, go to `shared/datasets/INFO`.If you would like to add a dataset, please also add the relevant metadata to the excel spreadsheet in the `shared/datasets/INFO` directory.

7.2 Gene signatures

Gene signatures derived from scRNA-seq datasets are also on the server home. You can find these under `shared/datasets/SIGNATURES`. The signatures are collapsed into a single .txt file in two different formats, "long" and "wide". The former includes metadata, while the latter contains the minimal amount of information - one signature per column of the matrix.

For more information on the gene signatures as well as how to add signatures, please see `shared/datasets/SIGNATURES/README`.

Chapter 8

Shared Code

The aim of the shared code is to implement the lab's core ideas on analysis of scRNA-seq data. The code is written in R and is publicly available via GitHub (see below). It was designed with a modular approach and hence is separated into several well-defined packages. The packages and functions are well documented and examples are provided within each package.

8.1 About the R packages

8.1.1 `scandal`

Source code | Report bugs

A framework that enables defining a single-cell experiment.

The package provides methods for loading the data, preprocessing and quality control, maintaining the data with a low memory footprint (using sparse matrices), various plotting methods, linking meta-data with expression data and more.

The package extends the `SingleCellExperiment` class, adapting it for use in our lab. See this tutorial for an introduction to the `SingleCellExperiment` class.

To install in R:

```
devtools::install_github("davishays/scandal")
```

8.1.2 infercna

Website & Tutorials | Functions index | Source code | Report bugs

Infer copy-number alterations from (single-cell) RNA-sequencing data.

The methodology implemented here was first formulated by Itay and colleagues during his postdoc Tirosh et al., 2014 and has been tried and tested in several publications since (Filbin et al., 2018; Neftel et al., 2019; Puram et al., 2017; Tirosh et al., 2016a, 2016b; Venteicher et al., 2017).

To install in R:

```
devtools::install_github("jlaffy/infercna")
```

Tutorial 1: Set your genome
Tutorial 2: Example with a scRNA-seq dataset

8.1.3 scrabble

Website & Tutorials | Functions index | Source code | Report bugs

Perform exploratory computational analyses on processed scRNAseq gene expression data.

The package focuses on unbiased methods in unsupervised clustering and dimensionality reduction to identify and characterize the transcriptionally-distinct subpopulations of cancer cells residing within tumours.

In its current implementation scrabble most closely reflects the methods implemented in Neftel et al., 2019 though any of the lab's papers should be useful as reference.

To install in R:

```
devtools::install_github("jlaffy/scrabble")
```

8.2 Contributing

Members of the lab to whom the shared code is relevant and of use are encouraged to contribute to it. This will both help the code to grow and develop but also allow you to fine-tune it for your own analyses. To make individual contributions, you can fork the project from GitHub. To contribute in a more long-term way, ask Julie or Avishay to add you as an official contributor to the GitHub package(s) in question.

Chapter 9

Experimental Protocols

A set of experimental protocols can be found in the Tirosh Lab dropbox organized by subcategory (link to folder). Please add your own protocols so this can become an improved and evolving resource for all experimentalists.

Chapter 10

Ordering

10.1 Weizmann warehouse orders

An ongoing list for the ordering of lab supplies from the Weizmann warehouse (machsan) is kept on the whiteboard outside Itay's office. Items ordered from the machsan include all disposables (i.e. tubes, gloves, pipettes), common/basic equipment (i.e timers, pipettors, lab coats), most tissue culture reagents (i.e. media, PBS), basic chemicals (i.e. ethanol, DMSO), and commonly used reagents (i.e. kits for qPCR, cDNA synthesis, RNA isolation). Warehouse orders are placed through the Tafnit system. Alissa regularly places orders but Rotem and Rony also have access to Tafnit (if anyone else would like access, please ask Michal Ovadia; note that the system is only available in Hebrew). When an item on the list is ordered, it should be marked with a checkmark. When the item arrives, it should be erased from the list. Orders from the machsan usually arrive within 3-4 days of being placed. If it is urgent, it is possible to call the warehouse and arrange to pick up an item in person from Piccioto after the order is received.

10.2 General ordering

All items that aren't ordered from the warehouse can be ordered with a price quote. In principle, some items from Sigma and Grafiti can also be ordered via Tafnit, though it is usually easier to order via price quote. Receiving a price quote requires knowing the local distributor of the product - many company websites will have a list of international distributors. Below, you will also find contact info for many of the most relevant distributors.

To receive a price quote, email the relevant distributor with the catalog number

and manufacturer. You can also ask how long it will take for the product to arrive if it's time sensitive (many products are ordered from abroad). Include in the quote request or in your email signature that you are from the Tirosh lab and lab address/departmental affiliation. You can forward price quotes to Natali (natalie.yikne@weizmann.ac.il) and she will place the order.

10.3 Office supplies

Graffiti is the office supply distributor for the Weizmann Institute. Their inventory/catalog can be found on their website. You can receive a price quote from liatji@graf.co.il.

10.4 Antibodies

In the Tirosh Lab dropbox, there is an excel file listing the details of the antibodies we have in the lab. Please consult it before ordering a new antibody and please update it when you receive a new antibody. Biocompare.com is a useful site for finding antibodies. You can filter your search with reactive species, conjugated fluorophores, and by application (IHC, flow, etc). There are many vendors of varying reputation for antibodies — please ask if you have questions about quality or which vendor to choose. For selecting fluorophores for FACS antibodies, the BD spectrum viewer is a helpful tool for selecting panels of antibodies without spectral overlap.

Below are some recommended antibody vendors and their local distributors.

For FACS antibodies:

- eBioscience (Rhenium), Biolegend (Enco), Miltenyi (Almog)

For immunohistochemistry/immunocytochemistry antibodies:

- Abcam (Zotal), R&D (Biotest)

For price quotes:

- Rhenium (eBioscience, Thermofisher) - Yaara Willensky yaara@rhenium.co.il
- Almog (Miltenyi) - Aviva Blechman Peretz aviva@almog.co.il
- Biotest (R&D) - Laora laora@biotest.co.il
- Zotal (Abcam) - Anna Bernstein anna@zotal.co.il
- Enco (Biolegend) - Yifat Ovadia - Yifat@enco.co.il

10.5 Molecular biology

Many popular molecular biology kits/products can be found in the warehouse (for qPCR we are typically using Applied Biosystems FAST SYBR green, for cDNA synthesis we like the Applied Biosystems high capacity cDNA kit, both available from the warehouse). For RNA isolation, Qiagen kits are available from the warehouse, though our favorite is the Zymo Quick RNA micro prep kit (Zotal is the distributor).

10.6 Growth factors

Please check the growth factors/cytokine spreadsheet in the Tirosh Lab dropbox to see inventory and please update when you receive something new. We have good experience ordering growth factors/cytokines from Peprotech located in Park HaMada (email ori@peprotechasia.com for quotes) and from Sino Biological (email Yifat Ovadia - Yifat@enco.co.il for a quote).

10.7 Miltenyi/MACS products

Almog is the supplier for Miltenyi products including reagents/disposables for the OctoMACS tissue dissociator, the dead cell removal kit, and MACS beads/reagants. Email aviva@almog.co.il or netta@almog.co.il for quotes. There is an option to either place an individual order or to join the monthly departmental order which saves money but takes longer. If your order is non-urgent, you can request the FOB price and ask Natali to add it to the monthly departmental order when you send her the quote.

10.8 Drugs

Cayman Chemicals is a reasonably priced and reliable supplier for drugs/compounds. Their distributor is Enco (Yifat@enco.co.il for quotes). Other vendors for drugs/chemical compounds include Sigma/Merck (shir.yohai@merckgroup.com), Molport (jose.garcia-tenorio@molport.com), and Selleck (manager@tivanbiotech.com).

10.9 Chemicals

Sigma/Merck is the largest distributor for chemicals. Many common chemicals can be ordered via the warehouse; Sigma price quote requests can be sent to

shir.yohai@merckgroup.com.

10.10 Primers

For primer design, everyone has their favorite program, but NCBI Primer BLAST is a good place to start. We order primers through IDT/Syntezza (idtdna.com). You can create your own user account with IDT on their site. Since primers are inexpensive and we tend to place small orders, we keep a standing order with IDT. In the payment order field, you can write 'Tirosh lab standing order'. You can email Chany Frankel (chany@syntezza.com) to confirm that there is money remaining in the standing order or to request to add money to the standing order via a price quote.

10.11 Illumina kits

Danyel Biotech is the distributor for Illumina sequencing kits (request a quote from sigal@danyel.co.il). The current kit in use for the NextSeq 500/550 is the NextSeq 500 high output kit (75 cycles) FC-4-4-2005.

Chapter 11

Weizmann Services

11.1 Internal Services

You can login to internal services using your Weizmann username/password through the Weizmann website on a computer connected to the Weizmann network or remotely with a VPN connection. Internal services functions as an ordering/payment system for most services within the Institute.

11.2 Printing Services

For printing services, including printing of posters, you can make the order in Internal Services. A PDF of the poster (or Dropbox link to PDF) can be sent to fiana.parente@weizmann.ac.il. It is also recommended to email or call Fiana to confirm receipt of the order. Poster templates can be found in the Tirosh lab dropbox folder. For more info on printing services, see here.

11.3 VPN connection

A VPN connection allows you to access the Weizmann network and the WEXAC server remotely. You can request a VPN connection through RSA soft token from Michal Ovadia. She will make the order to IT and IT will send further instructions.

11.4 Server (WEXAC) access/space

You can request WEXAC access through Michal Ovadia and she will submit the request to IT.

11.5 Cryostorage

11.6 Sending and Receiving Packages

Fedex shipments can be coordinated through Michal Ovadia. Our Fedex representative is Re'em Shpitzer (Rshiptzer@fdx.co.il, 054-560-6980) and our Fedex billing # is 579301163. Shipments of biological materials to/from abroad should include a declaration of essence and a customs declaration. If temperature sensitive, be generous with the dry ice.

11.7 FACS unit

You should visit the unit in person to register and coordinate training. Also, a safety form (to be renewed yearly) needs to be filled out and submitted for each user and can be accessed under 'FACS' in Internal Services. After-hours access through your I.D. card can also be arranged with the unit. Once you are a registered user, you can reserve equipment through Internal Services. Visit the FACS unit site for further info on the unit, including the instruments.

11.8 Sandbox unit

The Sandbox Unit is part of the Life Science Core Facilities located in the Levine Building. The unit offers researchers access and support for NGS-related technologies including library generation for bulk RNA-Seq and 10X 3' scRNA-Seq as well as other 10X products (i.e. ATAC-Seq). A 3-day workshop on generation of bulk RNA-Seq libraries with the bulk MARS-Seq protocol is offered regularly. Unlike traditional core units, you bring your samples and do the work yourself but all the materials and equipment are located there. Reservations for bench space and payment for use of supplies/reagants is via Genomics in Internal Services. Contact Merav Kedmi (merav.kedmi@weizmann.ac.il, x9212) or Hadas Keren-Shaul (hadas.keren-shaul@weizmann.ac.il) to arrange training, workshop attendance, and permissions. Additionally, there is a Sandbox Whatsapp group for coordinating shared sequencing runs (Hadas is admin).

There are also two NextSeq 500/550 sequencers in the unit. For training and permissions, please contact Muriel Chemla (muriel.chemla@weizmann.ac.il) in LSCF. Independent users can reserve sequencers through the Sandbox reservation system in Internal Services. Note that we are not charged for the sequencing run, only for the Illumina kit ordered through Danyel. Initiating a sequencing run also requires an account in susanc (ordered through the Bioinformatics Unit of LCSF, contact Irit Orr irit.orr@weizmann.ac.il x2470). See this link for SampleSheet templates/info required for initiating a sequencing run.

Chapter 12

Budgets

Budgets beginning with 71- correspond to external grants and budgets beginning with 72- correspond to internal grants. These budgets can be used for most scientific supplies and services. Abisch Frankel (budget 713381) is only for kits for scRNA-Seq and for sequencing. It is preferable to use external budgets first. Beyond that, priority is based on expiration date and money remaining (best to use budgets with closest expiration date and less money first). Equipment budgets begin with 4-. For questions concerning budgets, contact Elinor David (elinor.david@weizmann.ac.il, x4062).

Additionally, PhD students and postdocs have travel allowances. PhD students receive $2000 for travel following submission of their research proposal and $2250 following submission of their interim report. For postdocs, the annual allowance is $2000 yearly and the calendar year starts on 1 October.

Chapter 13

Equipment and inventory

An excel sheet of the equipment found in the lab is in the Tirosh Lab dropbox folder. Lumitron is the distributor who sold us the centrifuges and the PCR machine and they can be contacted for service (adi.s@lumitron.co.il, 073-2000777 for service requests). Getter is the supplier/service provider for the tissue culture hoods, the incubators, and the fridges/freezers (Yuval Borenstein, yuvalb@getter.co.il, 03-5761520 for service requests). Rhenium is the supplier/service provider for the microscope and Countess (Hadar Adler, Hadar@rhenium.co.il) and the FACS analyzer (tech.support@rhenium.co.il, 08-955-8855 for service requests). Hay Cohen (08-934-344, Wolfson 408) is the department administrator who oversees many equipment issues and contacting him is a good starting place for equipment concerns.

13.1 Office Supplies

Office supplies (pens, binders, notebooks, etc) can be found on the shelves and in the drawers located by the printer.

13.2 Lab supplies

Extra stocks of disposables (i.e. tissue culture plates and flasks, pipette tips, eppendorfs) can be found in the closet in the tissue culture room and in the closets with our lab name in the hall. These are good places to check first if you think we may be out of something.

13.3 Cytoflex (FACS analyzer)

Guidance and training on the Cytoflex can also be coordinated with Carmit Hillel-Karniel (carmit@rhenium.co.il). The sheath fluid consists of Millipore-grade water and 0.02% sodium azide. It can be either prepared by us or ordered through Rhenium. A deep clean with Contrad should be performed monthly. A cleansing agent with detergent is used for the daily clean. The blue tank is for sheath fluid and the yellow tank is for waste. We have 3 lasers (blue, red, violet) and the detector configuration can be viewed on the desktop of the Cytoflex computer. A word doc outlining Cytoflex daily maintenance and use is in the Tirosh Lab dropbox.

Chapter 14

Reading list

A folder for lab recommended papers and other useful resources (statistics,R, etc) is found on the lab dropbox at this link. The folder is organized by topics which reflect the main directions of the lab. The papers included are a short list of "core" papers that each lab member should read. Each new member of the lab should start by reading the relevant papers to them. Any lab member can recommend a paper to be added to the reading list, and this list is managed by the chosen lab member that is responsible. In addition, in the "R" folder, there is an excel sheet with recommended online resources.

Chapter 15

Lab safety and Waste disposal

15.1 General Safety

tl;dr x2999 or 08-934-2999 for emergencies and to report accidents (24/7)

All lab members are required to attend a safety training. The powers at be will likely contact you with a date to attend but if you choose to be proactive you can email safety.training@weizmann.ac.il for registration. :) A first aid kit is located on the top shelf by the coffee machine (red bag). An emergency shower and eyewash station as well as absorbent material for pouring on spills can be found immediately outside the door of the main lab. All accidents should be reported to x2999. Our annual lab safety plan (ASP) can be found in the Tirosh Lab dropbox.

See here for detailed safety information.

15.2 Waste disposal

Chemical and biological waste can be disposed of in the chemical and biological waste closet located in the opposite corridor on the first floor of Wolfson. Chemical waste containers are located in the cabinet above the sink in the main lab. Stickers for proper labeling are located in the waste closet. Bags for biological waste are in the bottom to the right of the sink in the main lab and in the bottom drawer in the tissue culture room. Tape the bag shut before placing it in the biological waste bin of the waste closet. Vacuum traps should be emptied

before the fluid level passes the line marked on them. They can be emptied into the sink but should be refilled with 10% volume of bleach after emptying.

15.3 Chemical hood

When working with hazardous chemicals, including Trizol and PFA, use the hood located next door to the main lab in the common equipment room. Please remember to clean up after yourself.

15.4 Safety equipment

Experimentalists should wear a lab coat, closed-toe shoes, and gloves when conducting experiments. A face mask and insulated gloves for liquid nitrogen are located in the main lab. Take special care when handling human samples and avoid use of sharps when handling these samples if possible. HBV vaccination is required for experimentalists handling human samples.

Chapter 16

Lab Maintenance

Ongoing chores and responsibilities to be divided among experimentalists include:

1. autoclave - preparation of materials (eppendorfs, Pasteur pipettes, H20) and transporting to/from autoclave on 3rd floor
2. waste disposal - bringing chemical and biological waste to the waste disposal closet (and replacing with new bags/containers), emptying vacuum traps in tissue culture and main lab and refilling with bleach
3. tissue culture maintenance - replacing water pans in the incubators and treating with Aquaguard (biweekly to monthly), replacing water in the water bath and treating with Aquaguard (monthly), autoclaving incubator racks (bi-annually), disinfection in case of a contamination, keeping the tissue culture room stocked with supplies
4. aliquoting of common reagents - aliquoting FBS, pen-strep, glutamine, Aquaguard 1&2 on a regular basis for the whole lab and keeping these reagents in stock.

Chapter 17

WEXAC (Weizmann EXAscale Cluster)

For more advanced help and information, please see the WEXAC slides on the lab dropbox.

17.1 Software

`module avail` lists all available software.
`module avail` lists software if it exists, or versions of it if they exist.
`module load` loads software.
`module ls` lists all software that you have currently loaded.

If you find yourself always loading the same modules, you may want to instead add the relevant module load command to your shell's configuration file in your home directory, e.g. to ~/.bashrc. You can create this file if it doesn't exist.

17.2 Adding software

Option 1: Email HPC@wexac.weizmann.ac.il with a request to add software.
Option 2: Compile software in your home directory. Put the executables in a sub-directory in your home and make sure to add the directory to the `$PATH` variable. E.g. `export PATH=~/bin:$PATH`, assuming you chose to place executables in `~/bin`. To permanently update the $PATH variable, add the export command to your `~/.bashrc`.

17.3 Database

Some data are used by several labs at Weizmann and as such WEXAC have put a significant amount of data under `/share/db`. You can check whether for example your desired genome sequence or index file already exists here before downloading it yourself.

17.4 Our server on WEXAC

Our server or 'compute node' is called `cn077`. It has ~200GB RAM and 1 core (Nov '19). `cn077` can be accessed directly or via one of four 'access nodes' (`access1`, `access2`, `access3`, `access4`) whose job it is to properly manage and distribute memory between lab members. For this reason, **if you're working interactively on the server you should primarily use one of the access nodes**. It shouldn't matter which of the four you use.

Access
access node: `ssh <username>@access4.wexac.weizmann.ac.il`
compute node: `ssh <username>@cn077.wexac.weizmann.ac.il`

17.5 Submitting jobs

If you are running something that requires substantial memory/time, you should submit this as a job to one of the queues on WEXAC (or sit back and wait to receive a call from HPC).

To name a few queues:
`tirosh`
`new-short`
`new-medium`
`new-long`
`new-all`

Which queue you choose can depend on a number of things: job duration & memory requirements, how busy a queue is and which compute node it is associated with. For example, the `tirosh` queue runs jobs on `cn077`. Since `cn077` is private, the queue is not busy (relatively speaking) and your job may start running sooner. On the other hand since `cn077` only has 1 core, a computationally-intensive job may finish more slowly than on a queue with multiple cores.

For more information on available queues:
`bqueues -a`

To submit a job:
`bsub -q <queue> <job>`

Jobs are by default allocated 1GB of RAM. If this is not enough:
`bsub -q <queue> -R "rusage[mem=XGB]" <job>`, or
`bsub -q <queue> -R "select[mem>XGB]" <job>`
where `X` is the (minimum) amount of memory you want to allocate, up to 150GB.

`bjobs` lists currently running and pending jobs.
`bkill <job ID>` kills job. Job ID is given in `bjobs` output.
`bpeek` to glance at job progress (`STDOUT`, `STDERR`). See also `-o` and `-e` flags in `bsub`.