Week 1: Advanced Bioinformatics

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MMG 3320

Spring 2024

Please call me either:

Dr. Rodriguez

or

Princess

My Background

- Bachelors in Biology
- Masters in Immunology and Microbial Disease
- Doctor of Philosophy in Cellular Molecular Biology
  - Epigenetics
  - Next Generation Sequencing
  - B cell biology

ST. LAWRENCE UNIVERSITY

ALBANY MEDICAL COLLEGE

The University of Vermont

Course Audience

- This course was designed for primarily an **undergraduate** audience with training in the biological sciences
- Students who have **no** formal training in the data or computer sciences
- Computer/data background be warned that you may find the class pace slow…*still*

My Approach

Yes, I will primarily teach you how to run bioinformatics software

With a focus on building bioinformatic skills for the purpose of extracting meaning from complex, large datasets

Reproducible: to the point where your work can be repeated by other researchers, and they can arrive at the *same* result

The decision to focus on sequencing data

Sequencing data is *abundant* and has *broad* applications

- Variant detection
- Transcriptome
- Protein-DNA interactions
- Methylation

Used the analysis of these data to hone bioinformatic skills

- Data management
- Communication
- Visualization

What I am keeping *from last semester (i.e. what you can expect)*

*"I think the **homework assignments and projects** were a really great way for me to be able to apply what I was learning."*
*"**During in–class activities**"*
*"The **final project** was my favorite part of the course. It helped us to engage with the coding, troubleshoot, and learn for ourselves!"*
*"Dr. Rodriguez also **made ample time** for students who were having difficulty with their code or had questions about it."*
*"…it seems as if you could get the **same amount of knowledge** from reading the powerpoints in your own time than attending lectures."*

Learning and expertise varies

Auditory learners, visual learners, while others learn- by-doing

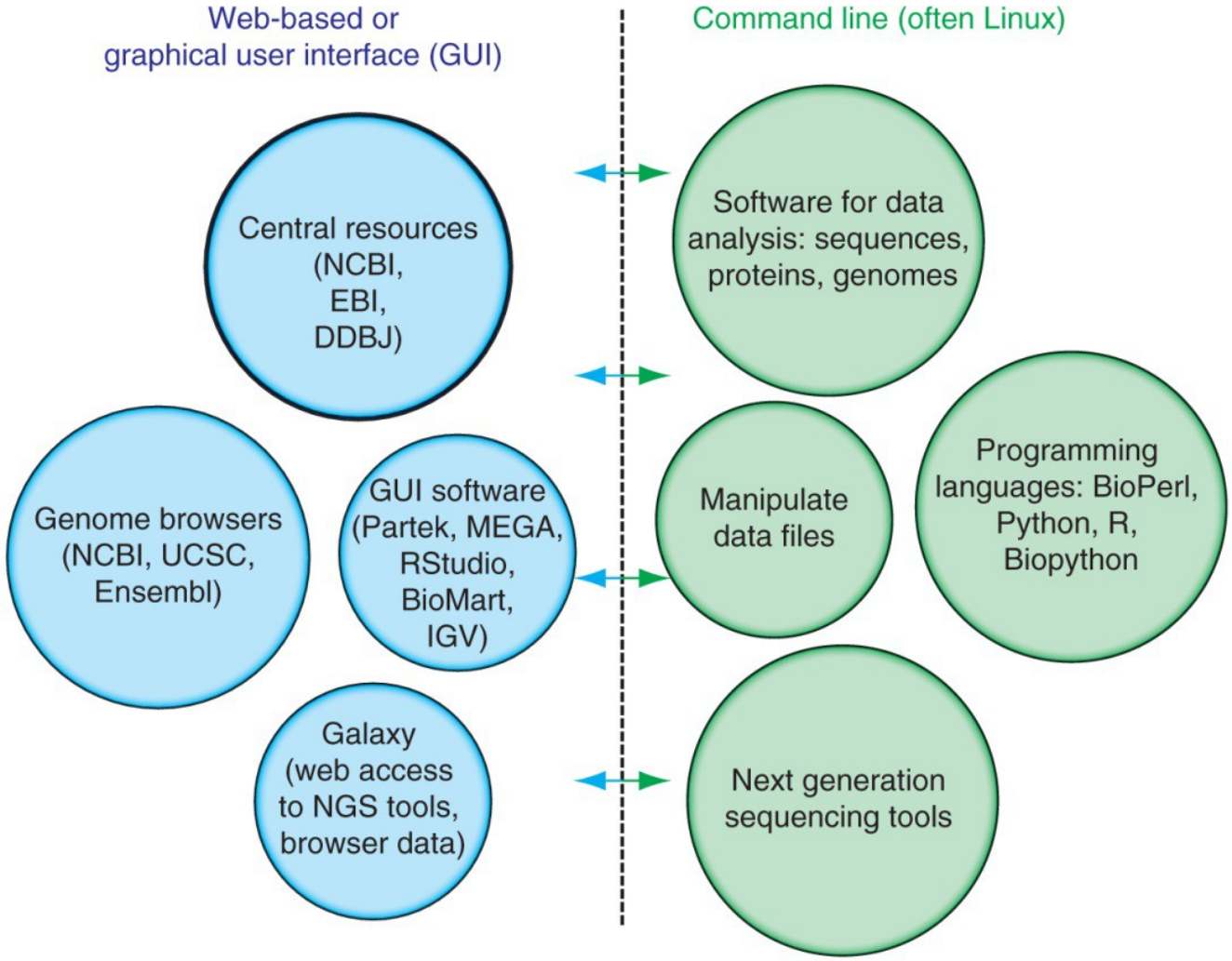
There are varying levels of expertise in this room.

How I am *trying* to improve this course

*"I just wish there was **more time** to go over the coding parts because if you got messed up then you were left behind."* *"I wish we had moved a **little slower** during the coding sections."*
*"**Include homework or small assignments** so that we are able to apply what we learn in class.." (coding)*
*"The R studio section **felt like way too much** for individuals without prior knowledge"*
*"I would have appreciated more time in class to actually work through **writing our own code** so that we could work through issues and better understand what each line does and how to build a working script."*

Let’s go over the syllabus

Two major approaches to bioinformatics



Tools are immediately accessible

Steeper learning curve

What is the command line?

- Underneath the Graphical User Interface (GUI) of your computer is the command line that runs your Operating System (OS)

- Working this way gives you access to internal controls, remote servers, and the ability to customize workflows (scripts)

- We access it with a shell (Terminal) which let's you give your computer commands via keyboard rather than a point and click

Command line + Bioinformatics

Why bother?

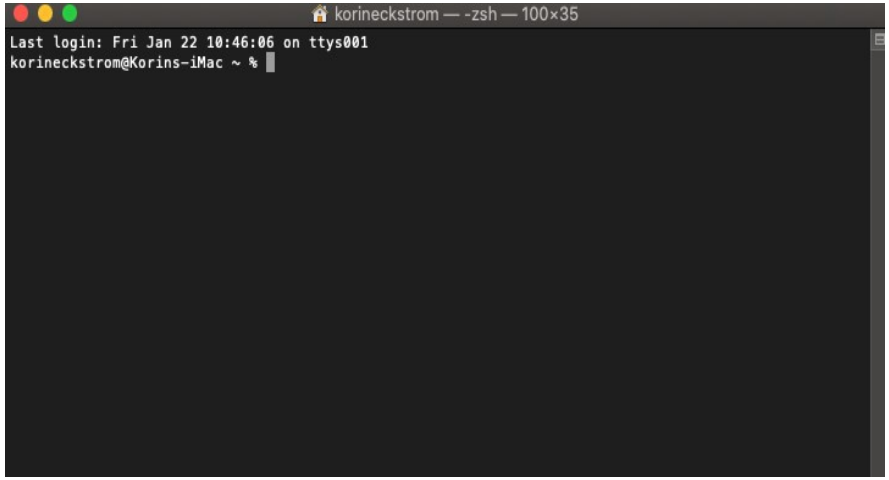
- GUI tools require memory just to run the interface, and most Bioinformatics applications are memory intensive to begin with or have additional flexibility on the command line
- Most of the time you will be working on a High Performance Cluster or remote server, as typical PCs do not have the required storage or compute power
- Reproducibility
- Ability to automate + create pipelines, or work with many files at once

Reproducibility

- Human error
- When we try to do the same thing 100 times we make mistakes
- A computer can perform the same task thousands of times without error
- Easier to communicate steps to others for reproducibility
- A different analyst re-performs the analysis with the same code and the same data and obtains the same result.

Topics we will cover:

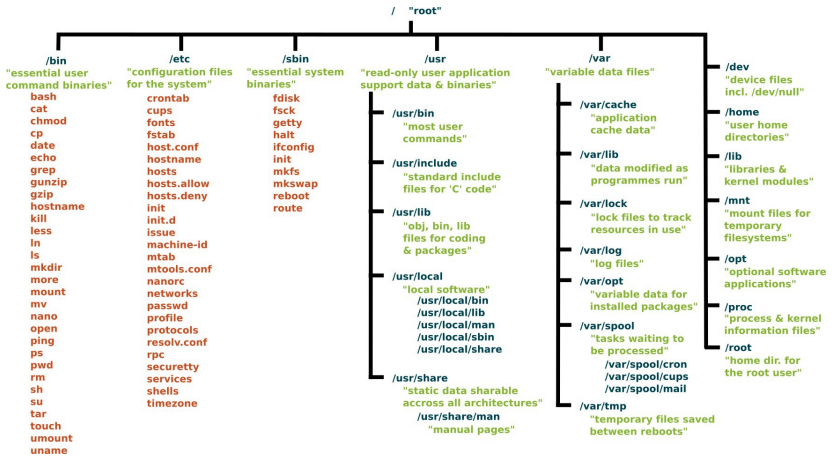
- How to access your shell



- Remote Servers & Benefits

Topics we will cover:

- Syntax = the “grammar” of a programming languages, needs to be **exact** for the computer to understand
  - Directories

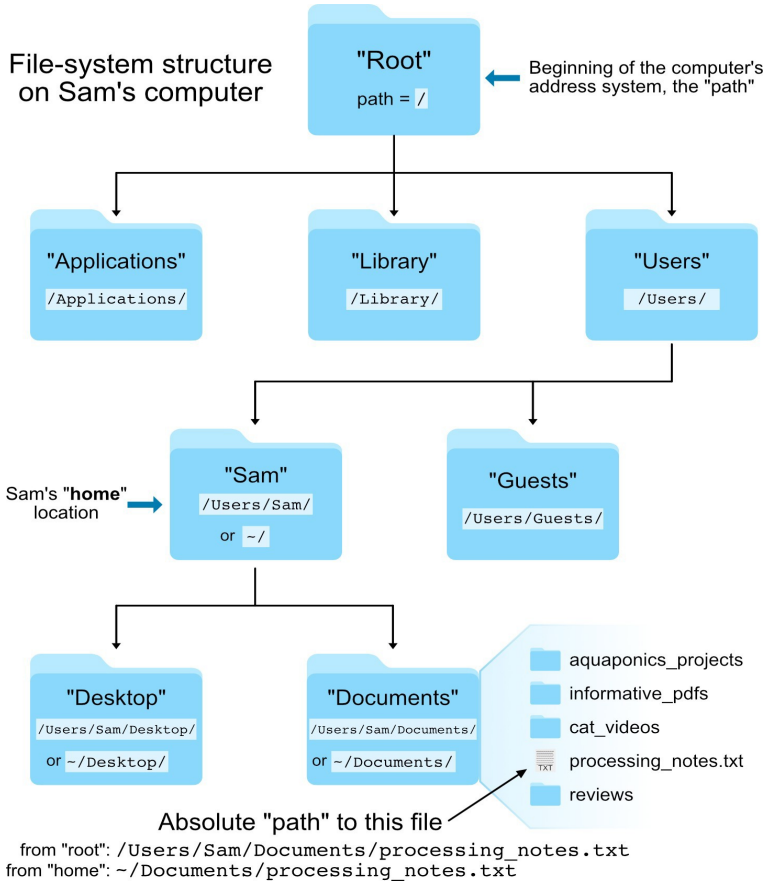


Topics we will cover:

PATH

Where am I? Where is that file?

- Can't click or drag and drop, and the computer can only find things if you tell it where they are
- PATH vs $PATH
- Absolute vs relative
- There are two locations all Unix-based systems share:
  - "Root" = where the address system of the computer starts
  - "Home" = where the user's location starts



Unix Getting Started

Topics we will cover:

Naming Files

- Case sensitive
  - File.txt ≠ file.txt
- Spaces without “” won’t be read correctly
- Adding dates can help with versions

GUI:

becomes….

```
/OneDrive\  -\ UVM\ Larner\ College\ of\ Medicine/
```

Topics we will cover:

Organization

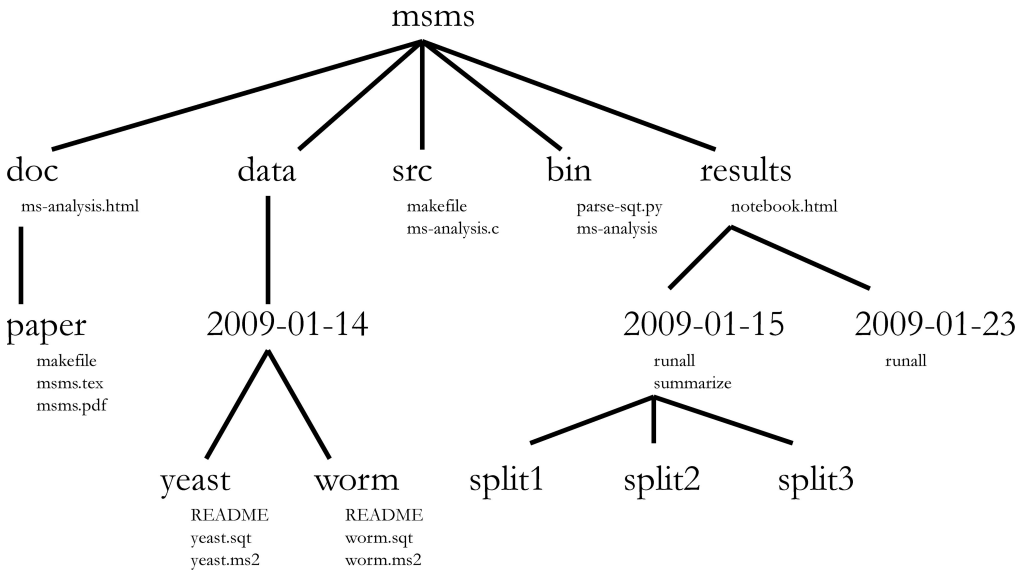
- Many pipelines don't just generate the final results file, but all of the intermediate steps it took to get there
- Having everything in one directory can not only be overwhelming, but can make automation that much harder
- Good organization can save you many headaches later on!
- You might do something like:
  - RawReads/
  - TrimmedReads/
  - Scripts/

```
samplename/
├── 0.basecall
│   ├── samplename.fq
│   └── nanoplots
├── 1.assemble
│   ├── samplename_merged.fasta
│   ├── samplename_raw_assembly.fa
│   ├── samplename_raw_assembly.fa.amb
│   ├── samplename_raw_assembly.fa.ann
│   ├── samplename_raw_assembly.fa.bwt
│   ├── samplename_raw_assembly.fa.fai
│   ├── samplename_raw_assembly.fa.pac
│   ├── samplename_raw_assembly.fa.paf
│   ├── samplename_raw_assembly.fa.sa
│   ├── assemble_100m (if specified)
│   └── assemble_250m (if specified)
├── 2.polish
│   ├── samplename_polished.corrected.fasta
│   ├── samplename_polished.fasta
│   ├── samplename_polished.fasta.bam
│   ├── samplename_polished.fasta.bam.bai
│   ├── samplename_polished.fasta.fai
│   ├── samplename_polished.fasta.misassemblies.tsv
│   ├── medaka (if specified)
│   ├── pilon (if specified)
│   └── racon (if specified)
├── 3.circularization
│   ├── 1.candidate_genomes
│   ├── 2.circularization
│   ├── 3.circular_sequences #circularized genomes
│   ├── 4.samplename_circularized.corrected.fasta
│   ├── 4.samplename_circularized.fasta
│   ├── 4.samplename_circularized.fasta.bam
│   ├── 4.samplename_circularized.fasta.bam.bai
│   ├── 4.samplename_circularized.fasta.fai
│   └── 4.samplename_circularized.fasta.misassemblies.tsv
└── 5.final
    ├── samplename_final.fa
    └── samplename_final.fa.fai
```

Topics we will cover:

Organization User Level

- If that's for an individual project, your VACC account might end up looking something like this
- Each new project gets a directory
- Versions or samples within these
- Tools or programs in one place that can be accessed from anywhere



Noble, PLoS Computational Biology 2019

Topics we will cover:

Submitting Jobs

Slurm scheduler

- If everyone tried to run their scripts at the same time, nodes would get mixed up, large jobs would take all the resources, and inefficiencies would be created
- To prevent this, HPCs run using "batch systems", which allows users to submit jobs requesting specific resources + with specific instructions in the form of a script
  - Instead of a single command at a time, you can submit whole workflows this way
- Knowledge Base: Run a job

```
#!/bin/bash
#SBATCH --partition=bigmem
#SBATCH --nodes=1
#SBATCH --ntasks=4
#SBATCH --mem=50G
#SBATCH --time=20:00:00
#SBATCH --job-name=racon_r1_metaflye
# %x=job-name %j=jobid
#SBATCH --output=%x_%j.out
# Notify me via email -- please change the username!
#SBATCH --mail-user=korin.eckstrom@med.uvm.edu
#SBATCH --mail-type=ALL
#
# change to the directory where you submitted this script
cd ${SLURM_SUBMIT_DIR}
#
# your job execution follows:
echo "Starting sbatch script myscript.sh at:`date`"
# echo some slurm variables for fun
echo "  running host:    ${SLURMD_NODENAME}"
echo "  assigned nodes:  ${SLURM_JOB_NODELIST}"
echo "  jobid:           ${SLURM_JOBID}"


cd /users/k/e/keckstro/scratch/working/directory/for/a/project
source activate ONT_tools

for i in /users/k/e/keckstro/scratch/working/directory/for/a/project/*_flye.fasta
do
  SAMPLE=$(echo ${i} | sed "s/_flye\.fasta//")
  echo ${SAMPLE}_flye.fasta
minimap2 -ax map-ont ${SAMPLE}_flye.fasta ${SAMPLE}_trimmed.fastq > ${SAMPLE}_to_draft.sam
racon ${SAMPLE}_trimmed.fastq ${SAMPLE}_to_draft.sam ${SAMPLE}_flye.fasta  > ${SAMPLE}_racon_r1.fasta
done
```


Let’s
make
sure we
can
login