

Welcome to The Carpentries Etherpad!

This pad is synchronized as you type, so that everyone viewing this page sees the same text. This allows you to collaborate seamlessly on documents.

Use of this service is restricted to members of The Carpentries community; this is not for general purpose use (for that, try <https://etherpad.wikimedia.org>).

Users are expected to follow our code of conduct: https://docs.carpentries.org/topic_folders/policies/code-of-conduct.html

All content is publicly available under the Creative Commons Attribution License:
<https://creativecommons.org/licenses/by/4.0/>

Zoom Link: <https://ksu.zoom.us/j/93785525724?pwd=Y3oGaRnj46OZZjCEeZOlFSQWnW8BaF.1>

Meeting ID: 937 8552 5724

Passcode: 629512

Do not connect to audio

Wireless: "KSU Guest"

Day 1: Attendees

Kendra Spahr - Academic Services Librarian, Kansas State University

David Molik - Computational Biologist, USDA ARS Arthropod-borne Animal Disease Research Unit (ABADRU)

Carol Sevin - Academic Services Librarian, Kansas State University

Gwendolyn Sibley - Scholarly Communications and Copyright librarian - Helper

Tom Misilo - Systems Administrator, Kansas State University

Carolyn Jackson Scholarly Communications & OER Librarian, K-State Libraries Center for Scholarly Publishing

Julia Eilert, KSU GRA in HWWGRU. Genetics masters program.

Ramgopal Prajapati, Postdoc, Plant molecular biology, Plant Pathology, K-State

Raissa Debacker - Postdoc, Plant Pathology/Kstate

Luiza Adami - Grad Student, Genetics/Plant Path, Kansas State University

Erin Schirtzinger - Research Assistant Professor, Diagnostic Medicine/Pathobiology, K-State

James Zhu USDA-ARS, Foreign Animal Disease Research Unit, NBAF

Patricia Assato - Research Associate, Diagnostic Medicine & Pathobiology, K-State

Amy Bernardo - USDA Genotyping Lab, HWWGRU

Raymond Deiser - Systems Librarian at Kansas State University. I maintain the libraries catalog and discovery layer (Search It -- https://k-state.primo.exlibrisgroup.com/discovery/search?vid=01KSU_INST:NewUI)

Rose Scott- Undergraduate Entomology - USDA-ARS Shults & Gerken labs - Interest in Arthropod borne diseases, genetics, and nonmodel species research. <https://www.linkedin.com/in/rose-scott/>

Sydney Andreano- VBS Graduate Student, department of Anatomy & Physiology in College of Veterinary Medicine at Kansas State University

Derek Samarian - Foreign Animal Disease Research Unit, NBAF

Neethu Francis- Visting Research Scholar, Genetics & Plant Breeding

Venkate

Dr Dave Turner - Beocat application scientist

Jacqueline Maille- Phd Student, Department of Entomology, Kansas State University

Ruolin Bian - Post Doc, Agronomy

Wei Zhao-visting scholar, Agronomy

Rupinder Singh -Phd Student, Department of Entomology, Kansas State University

Today's Setup

- Pre-workshop Survey - <https://carpentries.typeform.com/to/wi32rS?slug=2024-08-05-genomics-workshop>
- Download workshop files
 - Make a copy of the data file on your local system by going to <https://github.com/carpentries-incubator/life-sciences-workshop> and clicking 'Clone or download' followed by 'Download zip'. Extract the files in 'life-sciences-workshop-gh-pages\data' to a suitable directory
- make sure you have access to spreadsheet software
- make sure you have access to RStudio
- About BeoCat / SciNET
 - Dr. Dave Turner, K-State BeoCat (documentation:https://support.beocat.ksu.edu/Docs/Main_Page)
 - Rocky Linux with the slurm batch scheduler
 - Over 10k Interl/ADM cores on 310 compute nodes
 - 53 TeraBytes of RAM memory
 - Low-latency 30-100 Gbps InfiniBand/RoCE network
 - 1/10/40 Gbps Ethernet to a 1 PetaByte file server
 - 290 TB fast scratch space/fastscratch
 - 170 32-bit NVIDIA GPUs and 4 64-bit NCODOA P100s
 - 310 Compute Nodes access through Slurm
 - 4 Interactive nodes accessed only through OnDemand
 - BeoCat Group - any of them might answer support tickets
 - Director, Dan Andresen
 - Application Scientist, Dave Truner
 - Lead System Admin, Adam Tygar
 - System Admin, Nathan Wells
 - Tips
 - if you're accessing beocat on a computer that's not already logged into your beocat account, ssh `username@headnode.beocat.ksu.edu`
 - useful command: `kstat --help` (kstat is the monitoring tool, this command provides information about the jobs that are running, `kstat --me` will show your jobs)
 - use BeoCat when your computer is too slow or you don't have enough memory to run your work
 - Dr. David Molik, SciNet (<https://scinet.usda.gov/guides/>)
 - Need HPC like SciNet/BeoCat for bioinformatics tools that assemble genomes
 - slurm - queue manager
- Accessing BeoCat/SciNet
 - to login on a Mac, terminal ssh command
 - to login on a PC, PuTTY ssh command
 - SciNet: ssh login <https://scinet.usda.gov/guides/access/ssh-login>, first install smallstep or you can use open ondemand: <https://scinet.usda.gov/guides/access/web-based-login>

- BeoCat: ssh <k-state eid>@beocat.ksu.edu or you can use open ondemand: <https://support.beocat.ksu.edu/Docs/OpenOnDemand>
- A useful tool for windows machines: <https://mobaxterm.mobatek.net/>
- Globus - to transfer files between institution and HPC (BeoCat/SciNet) accounts
 - USDA, ORCID/PID card to login for 48 hours
 - K-State, <https://support.beocat.ksu.edu/Docs/Globus>

Life Sciences Workshopow to use best practices for managing your research data, along with case studies and examples to help you use these techniques. <https://guides.library.stanford.edu/data-best-practices>

- File naming conventions vary with operating systems (ex. space in WIndows is fine, in Linux any space becomes '/')
- Linux commands help sort by last modification date so no need to include the date in the name
- Use a folder to separate/protect raw data from report/presentation files (ex. separate folders for archive, literature, reports, processed data, and raw data)

Data Management: Meta-data

- some data repositories generate DOI (figshare, zenodo, osf, Ag Data Commons)
- to archive code - GitHub and Zenodo link-in will generate a DOI for your GitHub code

Raw data - protect it! Make it a read only file, analyze/make changes in a copy

Data Management and Planning

- Look for guidance from funding agencies ex. <https://www.nal.usda.gov/data/data-management-planning>
- dmptool.org

Spreadsheets

- Professor Daniel Lemire from the University of Quebec, in a popular blog post titled **‘You shouldn’t use a spreadsheet for important work (I mean it)’**, states that *“spreadsheets are good for quick and dirty work, but they are not designed for serious and reliable work”*.
 - prone to errors, not reproducible, not designed for advanced statistics/large amounts of data/scalability
 - <https://eusprig.org/research-info/horror-stories/>
- Guiding principles
 - save and don't edit raw data
 - metadata, could be a spreadsheet but don't use tabs in excel spreadsheets (.csv is non-proprietary/more accessible format doesn't have tabs)
 - consistent treatment of empty cells described in dictionary
 - one cell, one datum (don't mix data ex. location, age in one cell)
 - rectangular data - one observation (row) x one variable (column), non-rectangular might have additional information like other information and tables in some columns/rows
 - dates
 - color
 - validation > data tab > data tools

R Introduction

- 'wide' data - lots of columns and fewer rows
- Tidy or 'long' data - one column contains every value, remaining columns give context1
- R is a programming language great for open science publishing methods/graphics for researchers

- RStudio - application/interface for coding in R
- up arrow - go back through things you've previously run
- na is handled differently by different scientific packages (also good to check how different versions handle things like 'na')

R Function notes:

sample - Imagine you have a bag of marbles, and you reach in for a handful. That is what the code will do with a set of data, like vectors or values.

Numeric: Any number that is not an integer, basically it has decimal places.

c() is used to create matrices

If your R console changes to a +, then hit the ESCAPE button and it will return to >.

Rbind: is used to stack dataframes, and is dependent on the number of columns in two data frames

Rep: replicate. Used to replicate. Written as rep('value name', #) The # indicates how many times the value should replicate.

When you merge data frames, you have to indicate a value that it can merge from. It is best to pick a column header that is in all data frames you are combining.

You can use the \$ to access certain cells under a column header.

Tuesday 2024-08-06

Attendees:

Carolyn Jackson, Scholarly Communication & OER Librarian, Instructor

Tom Misilo, K-State Libraries

Sydney Andreano- GRA

Library Resources and Research Support Tools

<https://lib.k-state.edu/>

List of Library Databases: <https://guides.lib.k-state.edu/az/databases>

Request Reference Help - https://kstate.qualtrics.com/jfe/form/SV_3PExB7S1B05rA4B (Both K-State and community)

Lib Guides - <https://guides.lib.k-state.edu/>

O'Reilly (database with tech books/tutorials) @ K-State <https://go.oreilly.com/kansas-state-university>

O'Reilly @ Manhattan Public Library <https://links.mhklibrary.org/oreilly>

Manhattan Public Library also has LinkedIn Learning <https://links.mhklibrary.org/linkedin-learning>

Project Organization and Management

Howard Hughes Medical Institute “Making the Right Moves: A Practical Guide to Scientific Management for Postdocs and New Faculty” section on Data Management and Laboratory Notebooks.

<https://www.hhmi.org/sites/default/files/2023-10/making-the-right-moves-second-edition.pdf>

The Digital Curation Center maintains a list of metadata standards (<https://www.dcc.ac.uk/resources/metadata-standards/listand>) some that are particularly relevant for genomics data are available from the Genomics Standards Consortium (<https://www.gensc.org/pages/projects.html>).

FAIR Standards <https://www.go-fair.org/fair-principles/> FAIR data is Findable, Accessible, Interoperable, and Reusable.

Messy Data:

https://datacarpentry.org/organization-genomics/files/Ecoli_metadata_composite_messy.xlsx

https://raw.githubusercontent.com/datacarpentry/wrangling-genomics/gh-pages/files/Ecoli_metadata_composite.tsv

Library resources on R published since 2020:

- [https://k-state.primo.exlibrisgroup.com/discovery/search?query=sub,equal,R%20\(Computer%20program%20language\)&tab=Everything&search_scope=MyInst_and_CI&vid=01KSU_INST:NewUI&facet=searchcreationdate,include,2020%7C,%7C2024&offset=0](https://k-state.primo.exlibrisgroup.com/discovery/search?query=sub,equal,R%20(Computer%20program%20language)&tab=Everything&search_scope=MyInst_and_CI&vid=01KSU_INST:NewUI&facet=searchcreationdate,include,2020%7C,%7C2024&offset=0)
- [https://search.nal.usda.gov/discovery/search?query=sub,exact,R%20\(Computer%20program%20language\),AND&tab=LibraryCatalog&search_scope=MyInstitution&sortby=rank&vid=01NAL_INST:MAIN&facet=searchcreationdate,include,2020%7C,%7C2024&mode=advanced&offset=0](https://search.nal.usda.gov/discovery/search?query=sub,exact,R%20(Computer%20program%20language),AND&tab=LibraryCatalog&search_scope=MyInstitution&sortby=rank&vid=01NAL_INST:MAIN&facet=searchcreationdate,include,2020%7C,%7C2024&mode=advanced&offset=0)

Project organization and management - Lesson

<https://datacarpentry.org/organization-genomics/>

Thought this UNIX Tutorial would be worth sharing: <https://info-ee.surrey.ac.uk/Teaching/Unix/>

Introduction to the Command Line for Genomics - Lesson

<https://datacarpentry.org/shell-genomics/>

Glossary and reference links - <https://datacarpentry.org/shell-genomics/instructor/reference.html>

For this section you will need a directory called "shell_data"

For Scinet:

On both ceres atlas this directory is located at:

/90daydata/shared/david.molik/shell_data

You can copy these with the following command:

cp -R /90daydata/shared/david.molik/shell_data ~

For Beocat

The space then '~' at the end abcp -rp /homes/daveturner/shell_data ~ove will put the shell_data into your home directory

Alternatively:

You can also download the files directly:

```
wget -O shell_data.tar.gz https://figshare.com/ndownloader/files/14417834  
tar -xvf shell_data.tar.gz
```

<https://datacarpentry.org/shell-genomics/instructor/02-the-filesystem.html#relative-path-resolution>

Wednesday 2024-08-07

To move files for Genomics on USDA ARS systems:

```
cd ~  
cp /90daydata/shared/david.molik/dc_genomics.sif .
```

To move files for Genomics on K-State **beocat**:

```
cd ~  
  
cp -r /opt/beocat/shared/containers/dc_genmics.sif .
```

```
cd ~  
wget -O dc_workshop.tar.gz https://figshare.com/ndownloader/files/14502617  
tar -xvf dc_workshop.tar.gz -C dc_workshop --strip-components=1
```

Data wrangling:

<https://datacarpentry.org/wrangling-genomics/>
https://datacarpentry.org/wrangling-genomics/files/Ecoli_metadata_composite.csv

Running an interactive job on our resources:

```
srun --nodes=1 --time=8:00:00 --mem=1G --pty --overlap bash  
# this allocates a node for you to use, scient/beocat relevant documentation tells you how to add more  
resources
```

Running a container:

```
(ceres and atlas): module load apptainer  
# beocat does not need to load the module
```

Next we run fastqc

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif fastqc -h
```

but, you will run fastqc out of your directory:

```
apptainer exec /homes/<userid>/<optional>/dc_genomics.sif fastqc -h
```

KSU

```
apptainer exec /opt/beocat/shared/containers/dc_genomics.sif trimmomatic -h  
# you can also copy the container to your directory  
apptainer exec /homes/.YOUR_LOGIN_NMAE/dc_genomic.sif trimmomatic -h
```

(1) download adaptor:

```
wget raw.githubusercontent.com/timflutre/trimmomatic/master/adapters/NexteraPE-PE.fa
```

(2) KSU Beocat:

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif trimmomatic PE SRR2589044_1.fastq.gz  
SRR2589044_2.fastq.gz \ SRR2589044_1.trim.fastq.gz SRR2589044_1un.trim.fastq.gz \  
SRR2589044_2.trim.fastq.gz SRR2589044_2un.trim.fastq.gz \ SLIDINGWINDOW:4:20 MINLEN:25  
ILLUMINACLIP:NexteraPE-PE.fa:2:40:15
```

check your Trimmomatic run

```
ls SRR2589044*
```

human eye

```
ls SRR2589044* -l -h
```

Compression of file
gzip SRR2584863_1.fastq

USDA

```
for infile in *_1.fastq.gz  
do  
    base=$(basename ${infile} _1.fastq.gz)  
    apptainer exec /90daydata/shared/david.molik/dc_genomics.sif trimmomatic PE \  
    ${infile} ${base}_2.fastq.gz \  
        ${base}_1.trim.fastq.gz ${base}_1un.trim.fastq.gz \  
        ${base}_2.trim.fastq.gz ${base}_2un.trim.fastq.gz \  
        SLIDINGWINDOW:4:20 MINLEN:25 ILLUMINACLIP:NexteraPE-PE.fa:2:40:15  
done
```

KSU

```
for infile in *_1.fastq.gz  
do  
    base=$(basename ${infile} _1.fastq.gz)  
    apptainer exec /homes/YOUR_LOG_IN/dc_genomics.sif trimmomatic PE  
done
```

Download reference genome

```
curl -L -o data/ref_genome/ecoli_rel606.fasta.gz  
ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/017/985/GCA\_000017985.1\_ASM1798v1/GCA\_000017985.1\_ASM1798v1\_genomic.fna.gz$ gunzip  
data/ref_genome/ecoli_rel606.fasta.gz - ftp times out without downloading - FTP is  
closed at K-State for security reasons - can ask for individual site ftp to be opened ie
```

NCBI

KSU

cd data/ref_genome

Copy from the dorectory below:

wget -o ecoli_rel606.fasta.gz

ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/017/985/GCA_000017985.1_ASM1798v1/GCA_000017985.1_ASM1798v1_genomic.fna.gz- ftp times out without downloading (Works if following cd ~/dc_workshop & then mkdir -p data/ref_genome before using)
\$ gunzip data/ref_genome/ecoli_rel606.fasta.gz

Download small data

curl -L -o sub.tar.gz <https://ndownloader.figshare.com/files/14418248>

tar xvf sub.tar.gz - makes a sub directory in data directory

\$ mv sub/ ~/dc_workshop/data/trimmed_fastq_small - sub directory in data will disappear

Results note

mkdir -p results/sam results/bam results/bcf results/vcf

reference genome upzip

Go to reference genome directory

gunzip ecoli_rel606.fasta.gz

Index the genome

General information for container

usda

apptainer exec /90daydata/shared/david.molik/dc_genomics.sif

KSU

apptainer exec /homes/YOURLOGIN/dc_genomics.sif

apptainer exec /homes/daveturner/genomic_workshop/dc_genomics.sif

apptainer exec /opt/beocat/shared/containers/dc_genomics.sif

your command

bwa index data/ref_genome/ecoli_rel606.fasta

Aligner run

Add USDA or KSU specific dc_genomics.sif file PATH

For #USDA

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif bwa mem  
data/ref_genome/ecoli_rel606.fasta data/trimmed_fastq_small/sub/SRR2584866_1.trim.sub.fastq  
data/trimmed_fastq_small/sub/SRR2584866_2.trim.sub.fastq > results/sam/SRR2584866.aligned.sam
```

For KSU

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif  
bwa mem data/ref_genome/ecoli_rel606.fasta  
data/trimmed_fastq_small/SRR2584866_1.trim.sub.fastq  
data/trimmed_fastq_small/SRR2584866_2.trim.sub.fastq >  
results/sam/SRR2584866.aligned.sam
```

For August 08 , 2024 Notes:::

Sorting of BAM file by Co-ordinates

```
apptainer exec ../dc_genomics.sif samtools sort -o results/sam/SRR2584866.aligned.sorted.bam  
results/bam/SRR258486.aligned.bam
```

For USDA

Go to your terminal

Go to dc_workshop Directory

module load apptainer

General information for container command

```
apptainer exec YOUR-dc_genomics.sif COMMAND .....
```

Run this command

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif samtools sort -o  
results/bam/SRR2584866.aligned.sorted.bam results/bam/SRR2584866.aligned.bam
```

KSU

wGo to your terminal

Go to dc_workshop Directory

- # If you need the dc_workshop directory:
- cd ~
- wget -O dc_workshop.tar.gz <https://figshare.com/ndownloader/files/14502617>
- tar -xvf dc_workshop.tar.gz -C dc_workshop --strip-components=1
- # Copy container to your home directory
- cp /opt/beocat/shared/containers/dc_genomics.sif ~

General information for container command

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif
```

run this command

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif samtools sort -o  
results/bam/SRR2584866.aligned.sorted.bam results/bam/SRR2584866.aligned.bam
```

Learning about the BAM file

USDA

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif samtools flagstat  
results/bam/SRR2584866.aligned.sorted.bam
```

KSU

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif samtools flagstat  
results/bam/SRR2584866.aligned.sorted.bam
```

VARIANT CALLING

Step 1 --- Calculate read coverage

USDA

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif bcftools mpileup -O b -o  
results/bcf/SRR2584866_raw.bcf -f data/ref_genome/ecoli_rel606.fasta  
results/bam/SRR2584866.aligned.sorted.bam
```

KSU

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif bcftools mpileup -O b -o  
results/bcf/SRR2584866_raw.bcf -f data/ref_genome/ecoli_rel606.fasta  
results/bam/SRR2584866.aligned.sorted.bam
```

Step 2 --- Detect single nucleotide variant (SNV)

USDA

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif bcftools call --ploidy 1 -m -v -o  
results/vcf/SRR2584866_variants.vcf results/bcf/SRR2584866_raw.bcf
```

KSU375

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif bcftools call --ploidy 1 -m -v -o  
results/vcf/SRR2584866_variants.vcf results/bcf/SRR2584866_raw.bcf
```

Step 3: Filter and report the SNV variants in variant calling format (VCF)

USDA

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif vcfutils.pl varFilter  
results/vcf/SRR2584866_variants.vcf > results/vcf/SRR2584866_final_variants.vcf
```

KSU

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif vcfutils.pl varFilter  
results/vcf/SRR2584866_variants.vcf > results/vcf/SRR2584866_final_variants.vcf
```

FILTERING data (SRR2584866_variants.vcf vs SRR2584866_final_variants.vcf)

USDA

Before Filter

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif bcftools stats  
results/vcf/SRR2584866_variants.vcf | grep TSTV
```

After Filter

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif bcftools stats  
results/vcf/SRR2584866_final_variants.vcf | grep TSTV
```

KSU

Before Filter

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif bcftools stats  
results/vcf/SRR2584866_variants.vcf | grep TSTV
```

After Filter

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif bcftools stats  
results/vcf/SRR2584866_final_variants.vcf | grep TSTV
```

Explore the VCF format:

```
less -S results/vcf/SRR2584866_final_variants.vcf
```

Assess the alignment (visualization) - optional step

USDA

Index of bam file

```
exec /90daydata/shared/david.molik/dc_genomics.sif samtools index  
results/bam/SRR2584866.aligned.sorted.bam
```

Viewing with tvview

```
apptainer exec /90daydata/shared/david.molik/dc_genomics.sif samtools tvview  
results/bam/SRR2584866.aligned.sorted.bam data/ref_genome/ecoli_rel606.fasta
```

KSU

Index of bam file

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif samtools index  
results/bam/SRR2584866.aligned.sorted.bam
```

Viewing with tvview

```
apptainer exec /homes/YOURLOGIN/dc_genomics.sif samtools tvview  
results/bam/SRR2584866.aligned.sorted.bam data/ref_genome/ecoli_rel606.fasta
```

Viewing with IGV

OPEN A NEW terminal in your laptop (powershell in Dell and Terminal in MacBook/Linux)

make a Directory

```
mkdir ~/Desktop/files_for_igv
```

change to that Directory

```
cd ~/Desktop/files_for_igv
```

Copy your files here for Viewing in igv

USDA

For ceres

<SCINetID>=Firstname.Lastname (FOR ME siddhartha.kanrar)

sorted BAM file

scp

```
<SCINetID>@ceres-dtn.scinet.usda.gov:~/dc_workshop/results/bam/SRR2584866.aligned.sorted.bam .
```

Index sorted BAM file

scp

```
<SCINetID>@ceres-dtn.scinet.usda.gov:~/dc_workshop/results/bam/SRR2584866.aligned.sorted.bam.bai .
```

reference genome

```
scp <SCINetID>@ceres-dtn.scinet.usda.gov:~/dc_workshop/data/ref_genome/ecoli_rel606.fasta .
```

final Variant file

```
scp <SCINetID>@ceres-dtn.scinet.usda.gov:~/dc_workshop/results/vcf/SRR2584866_final_variants.vcf .
```

For atlas

sorted BAM file

scp

```
<SCINetID>@atlas-dtn.hpc.msstate.edu:~/dc_workshop/results/bam/SRR2584866.aligned.sorted.bam .
```

Index sorted BAM file

scp

```
<SCINetID>@atlas-dtn.hpc.msstate.edu:~/dc_workshop/results/bam/SRR2584866.aligned.sorted.bam.bai .
```

```
### reference genome
```

```
scp <SCINetID>@atlas-dtn.hpc.msstate.edu:~/dc_workshop/data/ref_genome/ecoli_rel606.fasta .
```

```
### final Variant file
```

```
scp <SCINetID>@atlas-dtn.hpc.msstate.edu:~/dc_workshop/results/vcf/SRR2584866_final_variants.vcf .
```

Scripts

use scripts to automate a commonly used set of commands

write scripts in editor like nano

#! shebang tells computer which interpreter to use

#!/bin/bash

comments

ex. apptainer

- #aliases container for cleanliness
- alias run_c="apptainer exec /homes/USER/dc_genomics.sif"

ex. shortcut for ls -la

- alias ll= "ls -la"

A script for running **fastqc**

- #!/bin/bash
- # "#" denotes a comment line
- # USDA ARS requires apptainer to be loaded
- echo "Loading apptainer"
- module load apptainer
- # Beocat does not require apptainer
- # Aliasing our container for cleanliness
- run_c="apptainer exec /90daydata/shared/david.molik/dc_genomics.sif"
- set -e
- cd /90daydata/shared/david.molik/dc_workshop/data/untrimmed_fastq/
- echo "Running FastQC ..."
- \$run_c fastqc *.fastq*
- mkdir -p /90daydata/shared/david.molik/dc_workshop/results/fastqc_untrimmed_reads
- echo "Saving FastQC results..."
- mv *.zip /90daydata/shared/david.molik/dc_workshop/results/fastqc_untrimmed_reads/
- mv *.html /90daydata/shared/david.molik/dc_workshop/results/fastqc_untrimmed_reads/
- cd /90daydata/shared/david.molik/dc_workshop/results/fastqc_untrimmed_reads/
- # This portion unzips the fastqc .zip files
- echo "Unzipping..."
- for filename in *.zip

- do)
- unzip \$filename
- done
- echo "Saving summary..."
- mkdir -p /90daydata/shared/david.molik/dc_workshop/docs
- cat */summary.txt > /90daydata/shared/david.molik/dc_workshop/docs/fastqc_summaries.txt
- R Code 8/8/24 :

https://figshare.com/articles/dataset/Data_Carpentry_Genomics_beta_2_0/7726454

```
data <- read.csv('/location/to/data/combined_tidy_vcf.csv')
```

OR

```
data <- read.csv(file.choose())
```

```
subset
```

```
subset <- data.frame(variants[,c(1:3, 6)])
```

```
alt_alleles <- subset$ALT
```

```
snps <- c(alt_alleles[alt_alleles == "A"],
         alt_alleles[alt_alleles == "T"],
         alt_alleles[alt_alleles == "G"],
         alt_alleles[alt_alleles == "C"])
```

```
factor_snps <- factor(snps)
```

```
plot(factor_snps)
```

```
ordered_factor_snps <- factor(factor_snps, levels = names(sort(table(factor_snps))))
```

```
plot(ordered_factor_snps)
```

```
SRR258863_variants <- variants[variants$sample_id == "SRR2584863",]
```

```
snp_positions_2 <- c("8762685", "66560624", "67545785", "154039662")
```

```
install.packages("BiocManager")
```

```
BiocManager::version()
```

```
BiocManager::install("vcfR")
```

```
install.packages("dplyr") ## installs dplyr package
```

```
install.packages("tidyr") ## installs tidyr package
```

```
install.packages("ggplot2") ## installs ggplot2 package
```

```
install.packages("readr") ## install readr package
```

```
library("dplyr")      ## loads in dplyr package to use
library("tidyr")      ## loads in tidyr package to use
library("ggplot2")    ## loads in ggplot2 package to use
library("readr")      ## load in readr package to use
```

```
variants <- read.csv("https://raw.githubusercontent.com/datacarpentry/genomics-r-intro/main/episodes/data/combined\_tidy\_vcf.csv")
```