# MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES BIOINFORMATICS WORKSHOP

***Presents***

## *INTRODUCTION TO BACTERIAL GENOMICS:*

### *Quality Control and Data Preprocessing*


**INSTRUCTED BY**

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*Department of Medical Microbiology and Infectious Diseases*

*University of Manitoba*

# INFORMATION FOR PARTICIPANTS

**All workshops are being recorded and posted to the MMID Bioinformatics Workshop – YouTube**

**For live Q&A, go to slido.com and use participant code #2583403**

# 2023 MMID Bioinformatics Workshop Schedule

| DATE | INSTRUCTOR | TOPIC |
|---|---|---|
| **March 2** | Grace E. Seo | Introduction to the 2023 MMID Bioinformatics Workshop |
| **March 9** | Grace E. Seo | Introduction to conda and tool installation |
| **March 16** | Grace E. Seo | Introduction to genomics and viral data analysis |
| **March 23** | Jill Rumore | Bacterial Genomics |
| **March 30** | Jill Rumore | Reference Databases |
| **April 6** | Taylor Davedow | Beginner's Guide to Phylogenetic Trees |
| **April 13** | Taylor Davedow | Introduction to tree visualization and annotation using ggtree |
| **April 20** | - | Bfx workshop: Bring your own dataset! |
| **April 27** | - | Bfx workshop: Bring your own dataset! |

***April 20 and April 27 in-person sessions are open to the public (up to 100 people)!***

***Work with your colleagues/friends to analyze data together.***

# SET UP WI-FI (IN-PERSON PARTICIPANTS)

1. ***Connect to UofM-secure (if you are a student or staff)***
   ***- Use your @myumanitoba.ca or @umanitoba.ca login and password***

2. ***Connect to UofM-guest***

**To access uofm-guest Wi-Fi:**

1. Ensure your wireless card is active and connected to the **uofm-guest** network.
2. Open your web browser (e.g. Google Chrome, Microsoft Edge, Firefox, etc.) and browse to any website. This should redirect you to the **Acceptable Use Agreement** page.
3. Review the Acceptable Use Agreement for the unsecured wireless.
4. Select **I Agree**.

# LEARNING OBJECTIVES

1. *Use a publically available dataset to:*
   I. *Assess general data quality using fastqc*
   II. *Filter out low quality reads using fastp*
   III. *Remove host sequence content using Bowtie2 and samtools*

2. *Assess assembled sequence data quality using checkM*

## DISCLAIMER

*To provide a basic working instruction, all tools will be run with default settings. HOWEVER, careful consideration of analysis parameters in the context of the research question should be taken into account when analyzing your own datasets, as default parameters do not always provide the most optimal result.*

# GETTING STARTED…

1. **Open the terminal and navigate to the conda_workshop folder on your desktop**

```
cd /mnt/c/Users/Username/Desktop/conda_workshop
```

2. **Make a new directory called Bacterial_Genomics**

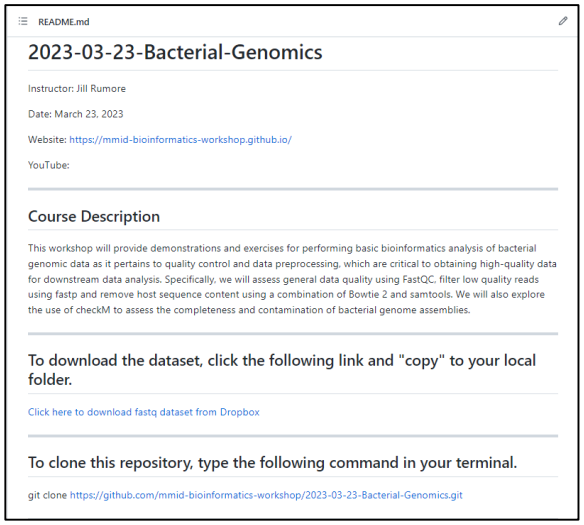
```
mkdir Bacterial_Genomics
```

**3. List the contents of the directory to confirm the new directory has been created**

```
ls
```

**4. Open your internet browser and navigate to the Bacterial Genomics Repository hosted on the MMID Bioinformatics Workshop Github**

https://github.com/mmid-bioinformatics-workshop/2023-03-23-Bacterial-Genomics



**5. Clone the 2023-03-23-Bacterial-Genomics repository in the Bacterial_Genomics directory**

git clone https://github.com/mmid-bioinformatics-workshop/2023-03-23-Bacterial-Genomics.git

**6. Download the fastq dataset to the Bacterial_Genomics directory**

https://www.dropbox.com/scl/fo/gx9ef004h5l537d58gk13/h?dl=0&rlkey=ouafrzefs7wv9nhbabzhhu9yb

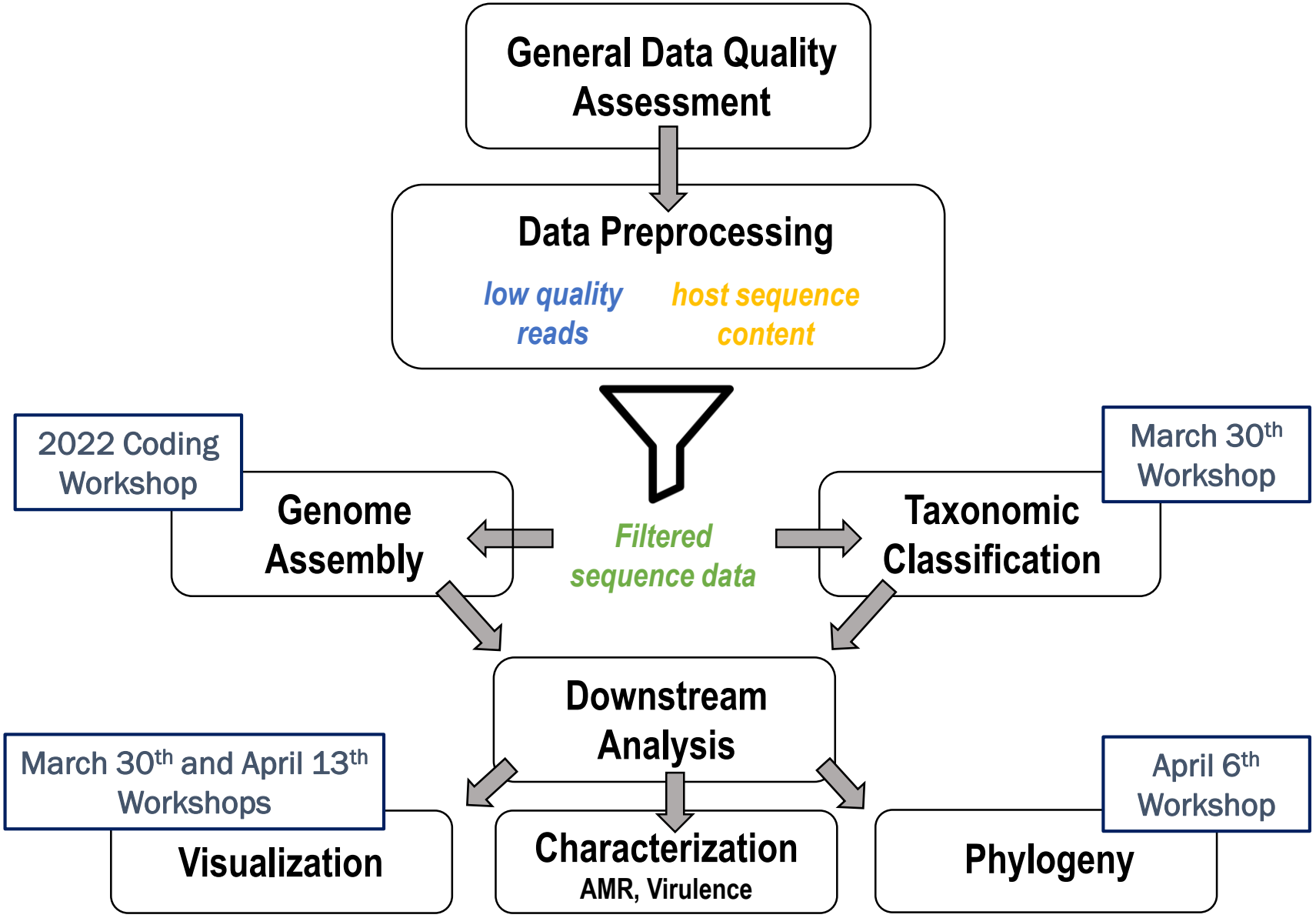
**7. Click on the link to download the Bowtie2 index from the MMID Bioinformatics Github repository to the Bacterial_Genomics folder**

https://www.dropbox.com/s/wrb253faf3i1bfo/Bowtie2Index.tar.gz?dl=0

**8. Unzip the fastq.zip folder than gunzip the individual fastq.gz files**

```
unzip fastq.gz
gunzip *fastq.gz
```

# BASIC WORKFLOW

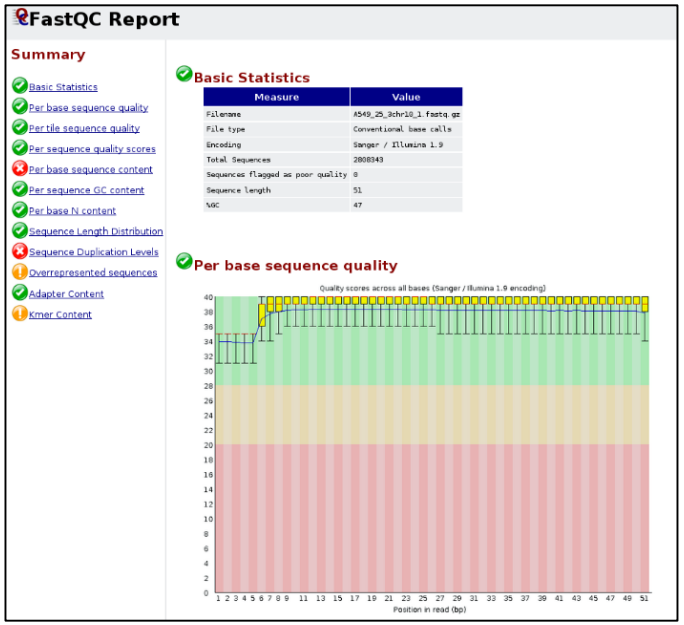


# GENERAL DATA QUALITY

## Sequence Analysis Viewer (SAV)


https://support.illumina.com/sequencing/sequencing_software/sequencing_analysis_viewer_sav.html

## FastQC

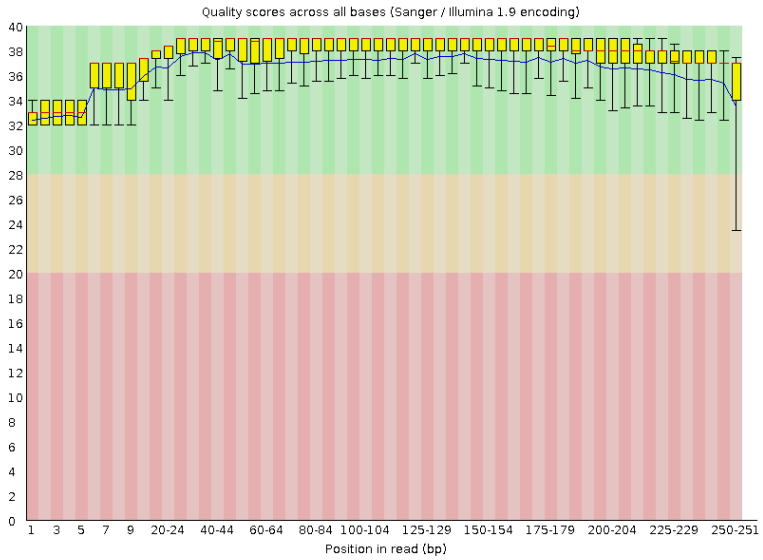



https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

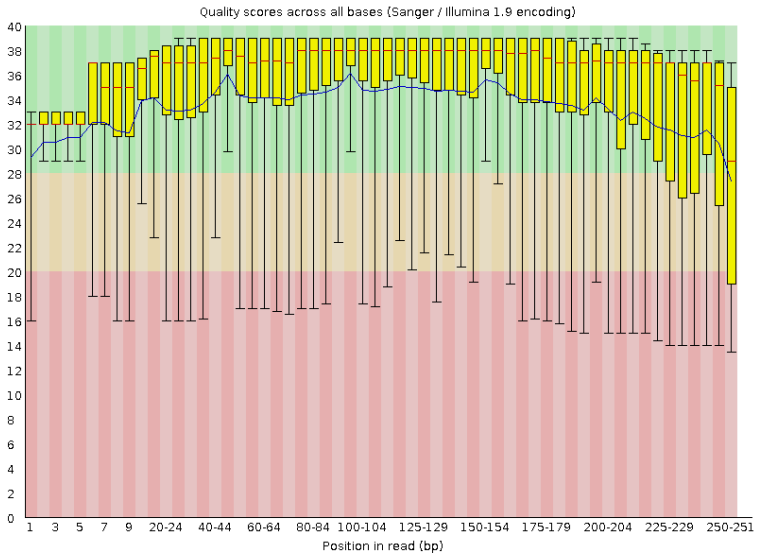
# FASTQC

## Per Base Sequence Quality

R1




R2

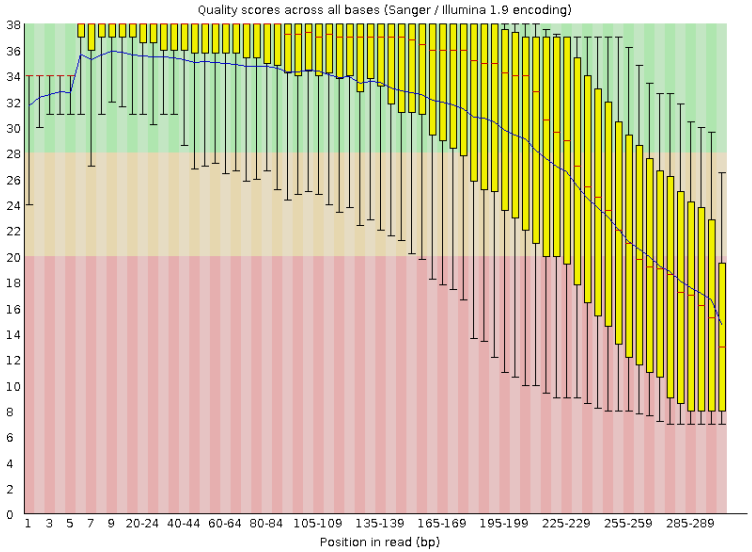



| QUALITY SCORE | PROBABILITY OF INCORRECT BASE CALL | INFERRED BASE CALL ACCURACY |
|---|---|---|
| 10 (Q10) | 1 in 10 | 90% |
| 20 (Q20) | 1 in 100 | 99% |
| 30 (Q30) | 1 in 1000 | 99.9% |

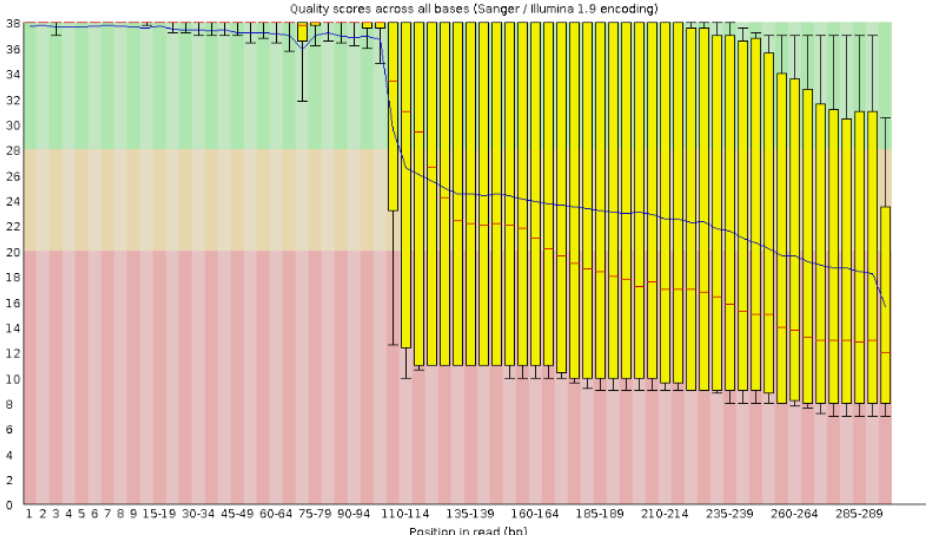
# FASTQC

## Per Base Sequence Quality

R1



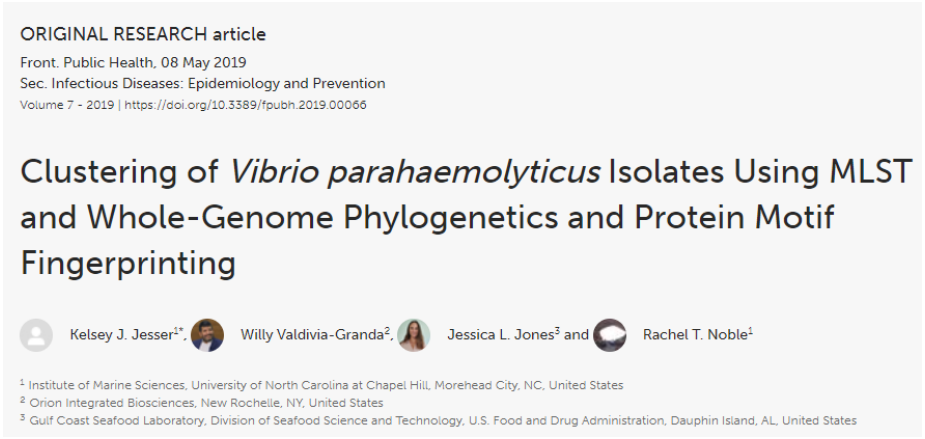

R2




Would this dataset pass the per base sequence quality check?

# PUBLICALLY AVAILABLE DATASET

https://doi.org/10.3389/fpubh.2019.00066



ORIGINAL RESEARCH article

Front. Public Health, 08 May 2019
Sec. Infectious Diseases: Epidemiology and Prevention
Volume 7 - 2019 | https://doi.org/10.3389/fpubh.2019.00066

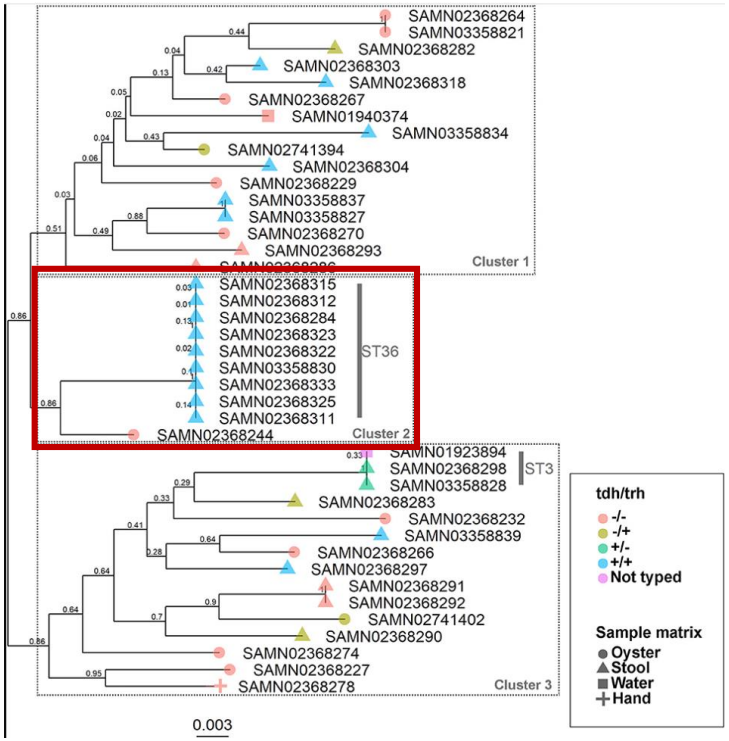
Clustering of *Vibrio parahaemolyticus* Isolates Using MLST and Whole-Genome Phylogenetics and Protein Motif Fingerprinting

Kelsey J. Jesser[1*], Willy Valdivia-Granda[2], Jessica L. Jones[3] and Rachel T. Noble[1]

[1] Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC, United States
[2] Orion Integrated Biosciences, New Rochelle, NY, United States
[3] Gulf Coast Seafood Laboratory, Division of Seafood Science and Technology, U.S. Food and Drug Administration, Dauphin Island, AL, United States

Uploaded to the MMID Bioinformatics GitHub Repository
https://github.com/mmid-bioinformatics-workshop

Data was sequenced using the Illumina HiSeq 2000



# DEMONSTRATION

# STEP BY STEP GUIDE

**1. Make a new directory called fastq in the Bacterial_Genomics directory**

```
mkdir fastq
```

**2. Move fastq files into the fastq directory**

```
mv *.fastq ./fastq
```

**3. Verify the fastq files have been moved**

```
cd fastq
ls
```

**4. Activate conda environment that contains the FastQC package**

```
conda activate conda_workshop
```

**5. Return to the Bacterial_Genomics directory**

```
cd ..
```

**6. Make a new directory called fastqc_reports**

```
mkdir fastqc_reports
```

**7. Run fastqc**

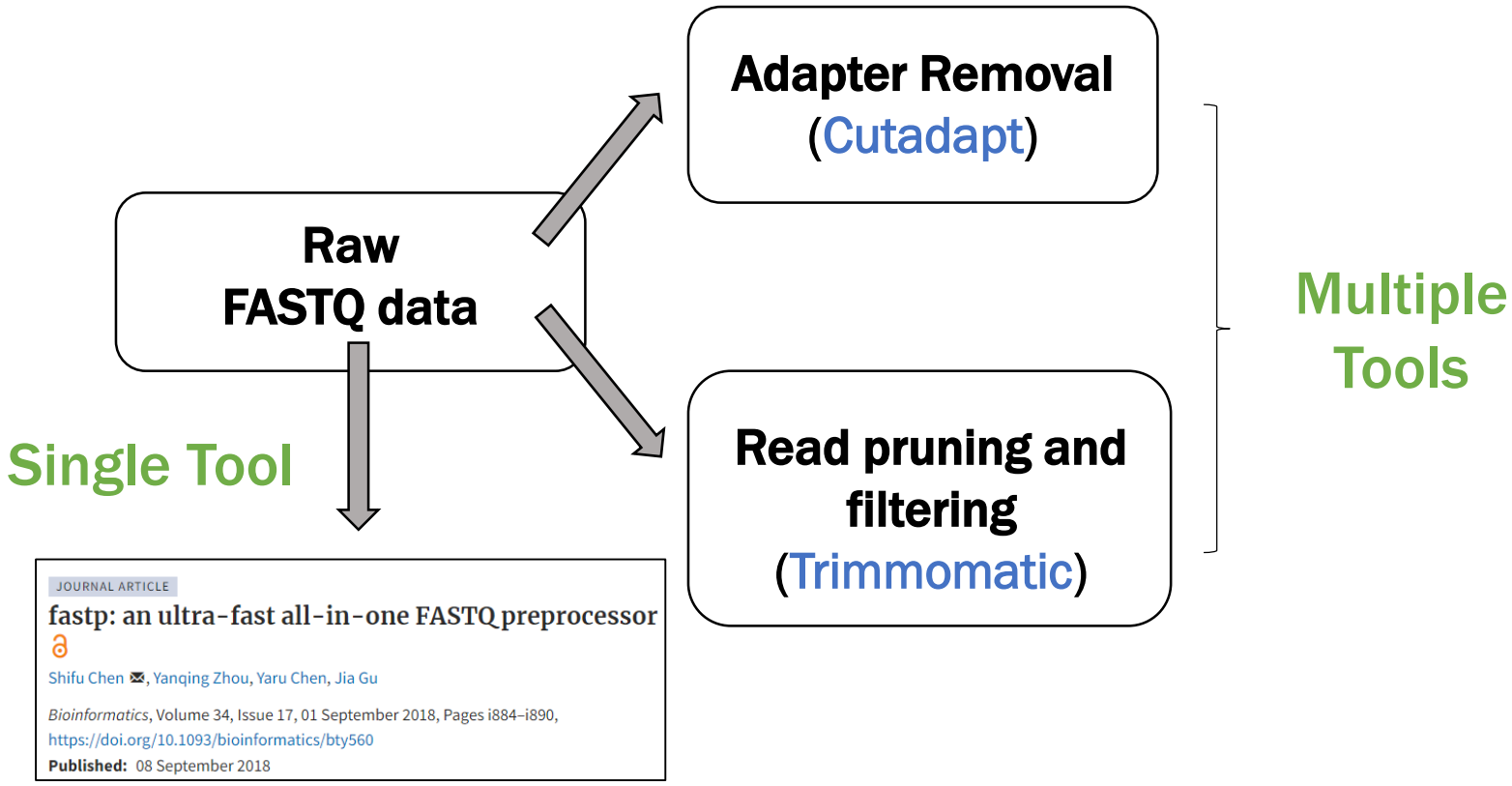
```
fastqc ./fastq/*.fastq -o ./fastqc_reports/
```

```
sbatch -c 1 --mem 2G -p NMLResearch --wrap="fastqc ./fastq/*.fastq
-o ./fastqc_reports“ (**Waffles Users Only**)
```

**IF WORKING ON THE CLUSTER (AKA WAFFLES) PLEASE USE THE SLURM WORKLOAD MANAGER WHEN SUBMITTING JOBS**

Detailed instructions can be found here:
https://github.com/MMID-coding-workshop/2022-01-19-Introduction-to-CONDA/blob/main/MMID_Coding_Workshop-IntroToConda_2022-01-19-Supplemental.pdf

# LOW QUALITY READ FILTERING




https://academic.oup.com/bioinformatics/article/34/17/i884/5093234
https://github.com/OpenGene/fastp

# fastp REPORT

## fastp report

### Summary

**General**

| | |
|---|---|
| fastp version: | 0.23.2 (https://github.com/OpenGene/fastp) |
| sequencing: | paired end (100 cycles + 100 cycles) |
| mean length before filtering: | 100bp, 100bp |
| mean length after filtering: | 98bp, 98bp |
| duplication rate: | 0.793924% |
| Insert size peak: | 119 |

**Before filtering**

| | |
|---|---|
| total reads: | 7.322614 M |
| total bases: | 732.261400 M |
| Q20 bases: | 699.351353 M (95.505697%) |
| Q30 bases: | 666.183279 M (90.976157%) |
| GC content: | 45.285202% |

**After filtering**

| | |
|---|---|
| total reads: | 6.890640 M |
| total bases: | 679.597300 M |
| Q20 bases: | 667.849881 M (98.271415%) |
| Q30 bases: | 639.829806 M (94.148374%) |
| GC content: | 45.087958% |

**Filtering result**

| | |
|---|---|
| reads passed filters: | 6.890640 M (94.100822%) |
| reads with low quality: | 431.518000 K (5.892950%) |
| reads with too many N: | 456 (0.006227%) |
| reads too short: | 0 (0.000000%) |

For more information on interpreting fastp output
https://github.com/MMID-coding-workshop/2022-01-26-Downloading-and-assembling-microbial-sequence-data

# DEMONSTRATION

# STEP BY STEP GUIDE

**1. Activate conda environment that contains the fastp package**

```
conda activate conda_workshop
```

**2. Make a new directory called fastp in the Bacterial_Genomics directory**

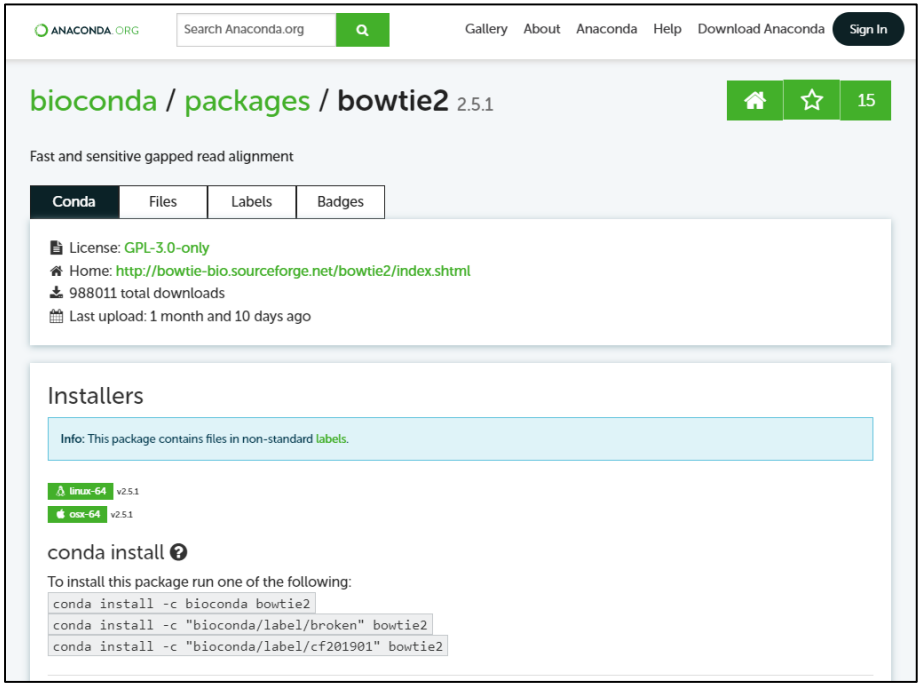
```
mkdir fastp
```

**3. Run fastp**

```
fastp -i ./fastq/SAMN02368311_R1.fastq -I
./fastq/SAMN02368311_R2.fastq -o ./fastp/SAMN02368311-fp_R1.fastq
-O ./fastp/SAMN02368311-fp_R2.fastq -h ./fastp/SAMN02368311.html
-j ./fastp/SAMN02368311.json
```

# HOST SEQUENCE FILTERING

# INSTALL TOOLS

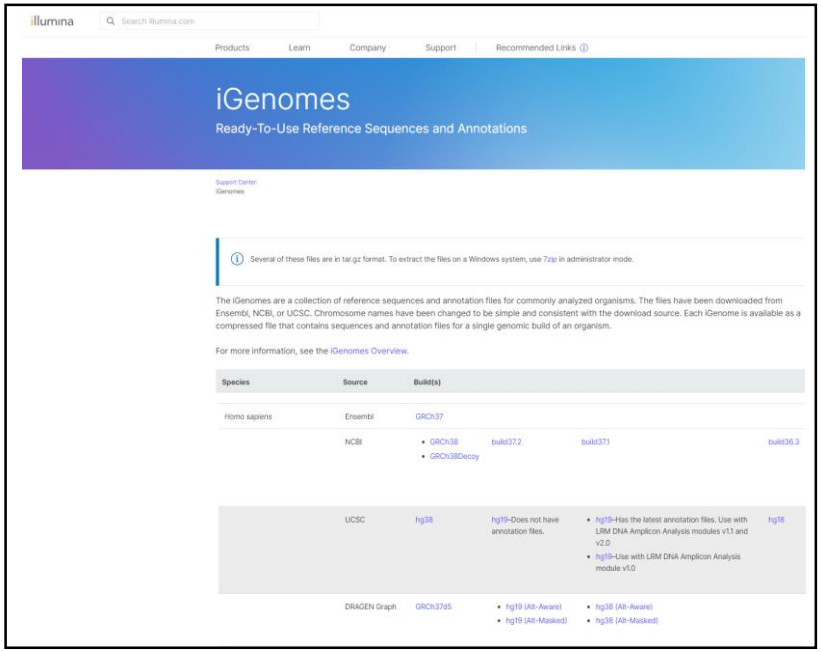



**1. Install the bowtie2 package in the conda_workshop environment**

conda install –y –c bioconda bowtie2

# BOWTIE2 INDICES

https://support.illumina.com/sequencing/sequencing_software/igenome.html




https://benlangmead.github.io/aws-indexes/bowtie

## Bowtie 2 indexes

Bowtie and Bowtie 2 are read aligners for sequencing reads. Bowtie specializes in short reads, generally about 50bp or shorter. Bowtie 2 specializes in longer reads, up to around hundreds of base pairs. HTTPS URLs allow you to download the files from your web browser or using command-line tools like `wget` or `curl`. The S3 URLs can be used with AWS tools, including the AWS console and AWS command-line interface.

In the past, Bowtie 1 & 2 had incompatible genome indexes. This changed in July 2019 when Bowtie v1.2.3 gained the ability to use Bowtie 2 formatted genome indexes (ending in `.bt2`). We list only Bowtie 2-format `.bt2` index files here.

You can download all the files for a given assembly as a single `zip` file, or as 6 separate `.bt2` files. For example, if you only need the forward version of the genome index (e.g. for exact matching only), you can download the files individually and omit the `.rev.1.bt2` and `.rev.2.bt2` files. Downloading already-decompressed index files might also be quicker for applications running in the AWS cloud.

| Species/Build | Source | HTTPS URLs | S3 URLs |
|---|---|---|---|
| Human / GRCh38 no-alt analysis set[1] | NCBI | full zip, .1.bt2, .2.bt2, .3.bt2, .4.bt2, .rev.1.bt2, .rev.2.bt2 | full zip, .1.bt2, .2.bt2, .3.bt2, .4.bt2, .rev.1.bt2, .rev.2.bt2 |
| Human / GRCh38 no-alt +decoy set[1] | NCBI | full zip, .1.bt2, .2.bt2, .3.bt2, .4.bt2, .rev.1.bt2, .rev.2.bt2 | full zip, .1.bt2, .2.bt2, .3.bt2, .4.bt2, .rev.1.bt2, .rev.2.bt2 |
| Human / GRCh38 + major SNVs | NCBI+1KG[2] | full zip, .1.bt2, .2.bt2, .3.bt2, .4.bt2, .rev.1.bt2, .rev.2.bt2 | full zip, .1.bt2, .2.bt2, .3.bt2, .4.bt2, .rev.1.bt2, .rev.2.bt2 |

# DEMONSTRATION

# STEP BY STEP GUIDE

**1. Make a new directory in Bacterial_Genomics directory called host_filtered**

```
mkdir host_filtered
```

**2. Decompress the Bowtie2Index.tar.gz file**

```
tar -xvzf Bowtie2Index.tar.gz
```

**3. Filter host reads using the iGenomes *Homo sapiens* bowtie2 index**

```
bowtie2 -x ./Bowtie2Index/genome -1 ./fastp/SAMN02368311-fp_R1.fastq
-2 ./fastp/SAMN02368311-fp_R2.fastq -S
./host_filtered/SAMN02368311.sam
```

**4. Move into the host_filtered directory and convert sam to bam**

```
cd host_filtered
samtools view -bS SAMN02368311.sam > SAMN02368311.bam
```

**5. Make a new directory called unmapped**

```
mkdir umapped
```

**6. Extract unmapped reads for both pairs**

```
samtools view -b -f 12 -F 256 SAMN02368311.bam >
./unmapped/SAMN02368311_unmapped.bam
```

**7. Move into the unmapped directory and make a new directory called sorted**

```
cd unmapped/
mkdir sorted
```

**8. Sort the bam file**

```
samtools sort -n -o ./sorted/SAMN02368311_sorted.bam --output-fmt
BAM SAMN02368311_unmapped.bam
```

**9. Move into the sorted directory and convert BAM file to fastq**

```
cd sorted/
samtools fastq -@ 2 -1 SAMN02368311-HR_R1.fastq -2 SAMN02368311-
HR_R2.fastq SAMN02368311_sorted.bam
```

# BACTERIAL GENOME ASSEMBLY

https://github.com/MMID-coding-workshop/2022-01-26-Downloading-and-assembling-microbial-sequence-data

README.md

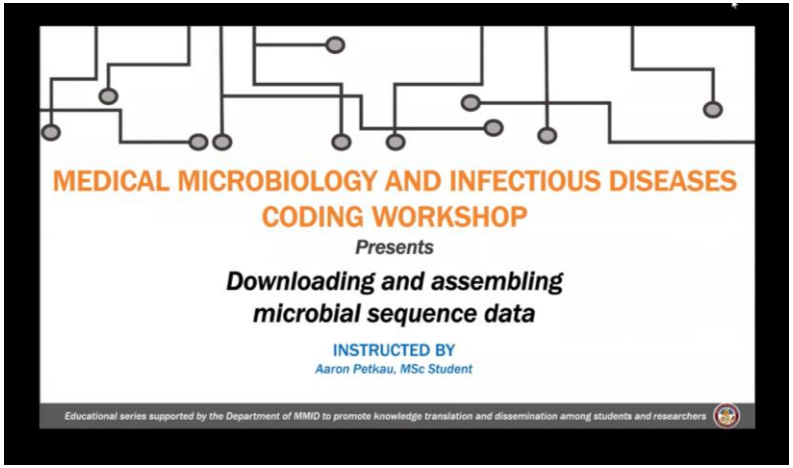
## Downloading and assembling microbial sequence data

launch binder

This repository contains the slides and additional materials for the **Downloading and assembling microbial sequence data** MMID Coding workshop for **January 26, 2022.**

Workshop lecture video (January 26, 2022) video is now available on YouTube at https://www.youtube.com/watch?v=qa0kqE8BIVQ

https://www.youtube.com/watch?v=qa0kqE8BIVQ



# ASSEMBLY QUALITY - checkM


https://github.com/Ecogenomics/CheckM/wiki

Genome Res. 2015 Jul;25(7):1043-55. doi: 10.1101/gr.186072.114. Epub 2015 May 14.

**CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes**

Donovan H Parks [1], Michael Imelfort [1], Connor T Skennerton [1], Philip Hugenholtz [2], Gene W Tyson [3]

Affiliations + expand

PMID: 25977477 PMCID: PMC4484387 DOI: 10.1101/gr.186072.114

Free PMC article

**Completeness**

*Estimated completeness of genomes as determined from presence/absence of marker genes*

**Contamination**

*Estimated contamination of genome as determined by the presence of multi-copy marker genes*

**Workflows**

***Lineage-specific*** – use this workflow to assess genomes from different taxonomic groups

***Taxonomy-specific*** – use this workflow to assess genomes from the same taxonomic group

# DEMONSTRATION

# STEP BY STEP GUIDE

**1. Make a new directory in Bacterial_Genomics directory called checkM**

```
mkdir checkM
```

**2. Navigate to the downloaded assemblies folder and decompress the fasta files**

```
cd 2023-03-23-Bacterial-Genomics
cd assemblies_skesa
gunzip *.gz
```

**3. Return to the Bacterial_Genomics folder**

```
cd ../../
```

**4. Run checkM taxonomy_wf on assembled data**

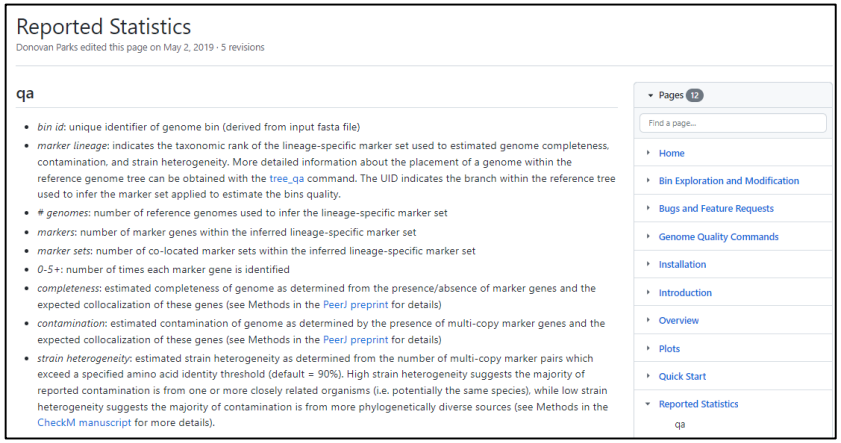
```
checkm taxonomy_wf genus Vibrio ./2023-03-23-Bacterial-
Genomics/assemblies_skesa/ ./checkM/ -t 2 -x fasta
```

**5. Run qa workflow**

```
checkm qa -o 2 -f ./checkM/checkM_quality.tsv --tab_table
./Vibrio.ms ./checkM/
```

# checkM RESULTS

https://github.com/Ecogenomics/CheckM/wiki/Reported-Statistics



Reported Statistics

Donovan Parks edited this page on May 2, 2019 · 5 revisions

qa

- *bin id*: unique identifier of genome bin (derived from input fasta file)
- *marker lineage*: indicates the taxonomic rank of the lineage-specific marker set used to estimated genome completeness, contamination, and strain heterogeneity. More detailed information about the placement of a genome within the reference genome tree can be obtained with the tree_qa command. The UID indicates the branch within the reference tree used to infer the marker set applied to estimate the bins quality.
- *# genomes*: number of reference genomes used to infer the lineage-specific marker set
- *markers*: number of marker genes within the inferred lineage-specific marker set
- *marker sets*: number of co-located marker sets within the inferred lineage-specific marker set
- *0-5+*: number of times each marker gene is identified
- *completeness*: estimated completeness of genome as determined from the presence/absence of marker genes and the expected collocalization of these genes (see Methods in the PeerJ preprint for details)
- *contamination*: estimated contamination of genome as determined by the presence of multi-copy marker genes and the expected collocalization of these genes (see Methods in the PeerJ preprint for details)
- *strain heterogeneity*: estimated strain heterogeneity as determined from the number of multi-copy marker pairs which exceed a specified amino acid identity threshold (default = 90%). High strain heterogeneity suggests the majority of reported contamination is from one or more closely related organisms (i.e. potentially the same species), while low strain heterogeneity suggests the majority of contamination is from more phylogenetically diverse sources (see Methods in the CheckM manuscript for more details).

Pages 12

Find a page...

Home
Bin Exploration and Modification
Bugs and Feature Requests
Genome Quality Commands
Installation
Introduction
Overview
Plots
Quick Start
Reported Statistics
qa

| A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|
| Bin Id | Marker lineage | # genomes | # markers | # marker sets | Completeness | Contamination | Strain heterogeneity | Genome size (bp) |
| SAMN02368311 | Vibrio (5) | 70 | 1084 | 381 | 100 | 0.13 | 0 | 5079511 |
| SAMN02368315 | Vibrio (5) | 70 | 1084 | 381 | 100 | 0.13 | 0 | 5079810 |

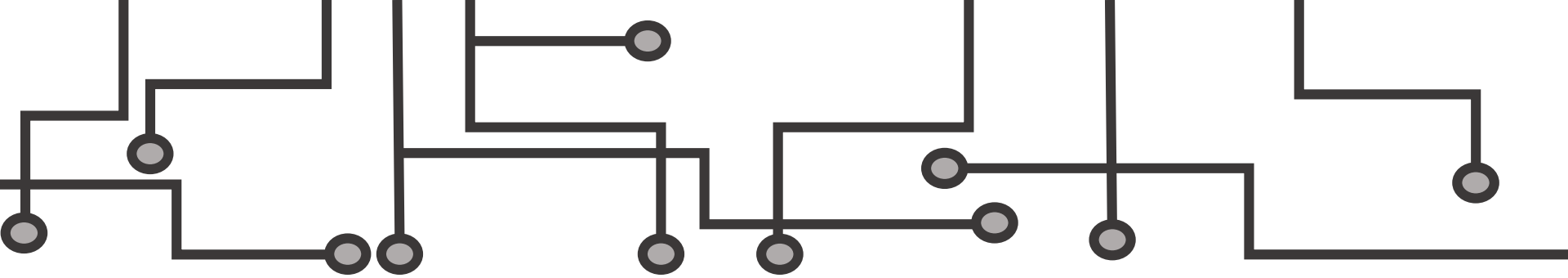
# HELPFUL RESOURCES

**How to change your password for sudo**
https://askubuntu.com/questions/931940/unable-to-change-the-root-password-in-windows-10-wsl

**Interpreting Quality Scores**
https://www.illumina.com/science/technology/next-generation-sequencing/plan-experiments/quality-scores.html

**SAM Flags**
https://broadinstitute.github.io/picard/explain-flags.html

**Bowtie2 documentation**
https://github.com/BenLangmead/bowtie2



# THANK YOU FOR ATTENDING!

***Please make sure to fill out the Exit Survey at https://docs.google.com/forms/d/e/1FAIpQLScNW1XUrnr16psbmW8yP3JTxlhWnVxp8n7ThwG3pBqdYbEXyQ/viewform?usp=sharing***

***We value your feedback!***

***More questions? Please email us at mmid.bioinformatics.workshop@gmail.com or post them to the workshop slack channel***