**Process raw Miseq reads and run them through Mothur QC pipeline and analysis script**

1. module load bcl2fastq/2.20

bcl2fastq -p 20 --create-fastq-for-index-reads -o <outputdir>

run the above bcl2fastq command in the Miseq raw reads output folder

(be sure to change the samplesheet.csv to a different name, so it can lump reads together and give us the 4 ‘Undetermined R1/R2/I1/I2 fastq files)

1. trim nextera adapter

cutadapt --pair-adapters

-j 20

-a CTGTCTCTTATACACATCTCCGAGCCCACGAGAC

-A CTGTCTCTTATACACATCTGACGCTGCCGACGA

-o Undetermined\_S0\_L001\_R1\_001.fastq.trim.gz

-p Undetermined\_S0\_L001\_R2\_001.fastq.trim.gz

Undetermined\_S0\_L001\_R1\_001.fastq.gz

Undetermined\_S0\_L001\_R2\_001.fastq.gz

1. Mothur QC

python3 pipeline.py -c settings.ini

Need to make sure that:

1. Settings.ini is properly set up

Example: */scicomp/groups/OID/NCEZID/DFWED/EDLB/projects/CIMS/HMAS\_QC\_pipeline/M347\_21\_026/settings.ini*

1. mothur\_py is installed
2. Mothur is on path (1.46.0)
3. cutadapt is on path
4. correct oligos file is set up
5. Run analysis script

python3 parse\_count\_table\_confusion\_matrix.py

-c final.full.count\_table (from Mothur QC)

-f fina.fasta (from Mothur QC)

-r reference.fasta

-s sample.csv

-o output.file

Need to have blast loaded: ml ncbi-blast+/LATEST

**Download SRA files and assemble isolate WGS**

1. Dowload sequence data files using SRA toolkit
   * 1. Installation

wget --output-document sratoolkit.tar.gz <https://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/current/sratoolkit.current-ubuntu64.tar.gz>

* + 1. Configure and set it up correctly
    2. fasterq-dump --split-files SRR1616822

1. Use Shovill to assemble
   * 1. ml shovill
     2. might need to cd to the home folder in order to use shovill (o/w complaining can’t read current work directory)
     3. shovill -R1 t3pio/Code\_Repository/T3Pio\_Main/DesignSet/SRR1616822\_1.fastq -R2 t3pio/Code\_Repository/T3Pio\_Main/DesignSet/SRR1616822\_2.fastq --outdir SRR1616822\_2014K\_0527\_skesa\_trim --assembler skesa --trim ON --cpus 40