

Post-industrial_modern_analysis-FS

- Apart from coverage, collecting data for post-industrial and modern analysis was done manually.
 - Using AMR++ outputs for post-industrial and modern data, genes under 100 reads were filtered out and entered into an excel sheet.
 - Cmd + F function was used to count genes and were then made into percentages.
 - Percentages were converted into a bar chart (**PostVsModern-Coverage_graph**; **Figure 4**).
- Coverage was calculated using in the following steps:
 - A csv file ('postIndModern50TArgs.csv') contained the required metadata, with one column naming the period (i.e. modern or post-industrial), MEG number, ERR accession (sample), and read count.
 - Two directories 'modern' and 'post-Industrial' containing sample fastq files were created.
 - A python script was made to create multiple scripts that used the information from the metadata to map individual BAM files to their MEGARes reference genes and calculate coverage:

```
amr_metadata = '/storage02/or-microbio/coverage_mapping_new/sbatch_job_scripts/postIndModern50TArgs.csv'
fixed_location = '/storage02/or-microbio/'
AMR_directory = '/storage02/or-microbio/AMR_download_genes/'

location_dict = {'post-Industrial': '/storage02/or-microbio/coverage_mapping_new/post-Industrial',
                 'modern': '/storage02/or-microbio/coverage_mapping_new/modern'}

with open(amr_metadata, "r") as meta_in:
    for line in meta_in:
        if not line.startswith('grouping'):
            line = line.split(",")
            if line[0] in location_dict:
                with
open("{0}_{1}_{2}.sbatch".format(line[0].strip(), line[1].strip(), line[2].strip()), "w") as
script_out:
    script_out.write("""#!/bin/sh

#SBATCH --job-name=AMR_map\n
#SBATCH --account=43070_203920\n
#SBATCH --partition=compute\n
#SBATCH --cpus-per-task=10\n
#SBATCH --time=2:00:00\n

module purge\n
module load BWA\n
module load SAMtools\n
\n
bwa mem -v 3 -t $SLURM_CPUS_PER_TASK -reference {0}{1}.fasta {2}/{3}_fixed_R1.fastq.gz
{2}/{3}_fixed_R2.fastq.gz | \n
samtools sort --threads $SLURM_CPUS_PER_TASK --reference {0}{1}.fasta -o
{4}coverage_mapping_new/{5}/{1}_{3}_mapped.bam\n
cd {4}coverage_mapping_new/{5}\n
samtools coverage -o {1}_{3}_coverage_table.tab {1}_{3}_mapped.bam\n""".format(AMR_directory,
line[1].strip(), location_dict[line[0]], line[2].strip(), fixed_location, line[0].strip()))
```

- This script outputted individual coverage tables which were collated using another python script:

```
import glob

print("period, gene, sample, Nreads, coverage")
for files in glob.glob("/storage02/or-microbio/coverage_mapping_new/**/*_coverage_table.tab",
recursive = True):
    details = files.split("/")
    gene, sample = "{0}_{1}".format(details[5].split("_")[0], details[5].split("_")[1]),
details[5].split("_")[2]
    with open(files, "r") as f_in:
        for line in f_in:
            if not line.startswith("#rname"):
                line = line.split("\t")
                print("{0},{1},{2},{3},{4}".format(details[4], gene, sample,
line[3], line[5])) (base) [fjst(ba(b((((((((((((base) [fjstande@login01 (Bradford-HPC)
coverage_mapping_new]$ cat collate_coverage.py
import glob

print("period, gene, sample, Nreads, coverage")
for files in glob.glob("/storage02/or-microbio/coverage_mapping_new/**/*_coverage_table.tab",
recursive = True):
    details = files.split("/")
    gene, sample = "{0}_{1}".format(details[5].split("_")[0], details[5].split("_")[1]),
details[5].split("_")[2]
    with open(files, "r") as f_in:
        for line in f_in:
            if not line.startswith("#rname"):
                line = line.split("\t")
                print("{0},{1},{2},{3},{4}".format(details[4], gene, sample, line[3], line[5]))
```

- Data from this coverage table was copied and pasted into Excel and converted into a box and whisker plot ([Figure 5](#)).