**Instructions for Use  
Image Analysis**This code is designed for any 96-well format spot-on-lawn experiment.

1. Copy the “Template for Image Analysis” folder, note that it contains some example data
2. Open “auto\_ZOI\_analysis” in MATLAB
3. Set imagedir (line 10) and params (lines 44-52) according to your imaging setup
4. See comments in code for how to skip specific steps of the analysis by listing filenames (useful for pausing partway through analysis or rerunning specific steps)

**Interaction Analysis & Plotting**This code isdesigned to generate the plots in the manuscript. While some functions may be helpful for analyzing the results of the “Image Analysis” code for new experiments, it has a lot of manuscript specific datasets and dependencies that will not be relevant for your analysis. It is provided for users to explore the data in the manuscript

1. Copy the “Interaction Analysis” folder
2. Set parameters in lines 13-52
3. Choose which plots to generate by setting a unique nonzero fignum for desired plots

**Bioinformatic Analysis**Thesepipelines are designed to work onthe MIT Engaging computing cluster (a SLURM system), they will need significant modifications before running on your system. Pre-computed results and links are available in the “local\_pangenome\_enrichment\_analysis” folder. Pre-computed tables used for plotting can be found in the “Interaction Analysis/data\_for\_code” folder.

1. Download raw isolate reads
2. Run snakemake\_assembly\_annotation pipeline (see readme\_pipeline\_instructions.txt in folder)
3. Run snakemake\_gainloss\_analysis pipeline (see readme\_pipeline\_instructions.txt in folder)
4. Run snakemake\_pangenome\_and\_BGC pipeline (see readme\_pipeline\_instructions.txt in folder)
5. Download specified results to local\_pangenome\_enrichment\_analysis
6. Run extract\_consensus\_proteins.py
7. Submit consensus\_proteins.faa to reference database searches (e.g. VFDB)
8. Run calculate\_pangenome\_enrichment.py
9. Optional: run snakemake\_breseq\_lineage37