# Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

# A lesson in the value of thorough QC/QA as it applies to whole genome sequencing

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# Background

- Started routinely using MagNA pure LC for extraction Spring 2018
- Including a negative control on each extraction run.
  - Nanodrop
  - Qubit
- By mid August we implemented a bioinformatics pipeline to evaluate the quality of a sequenced isolate.
  - FastQC
  - Serotype
  - Contigs

# Problem

- Specimens were exhibiting a higher than expected number of contigs (255-3173)
- Size range varied greatly between 2.7Mb-5.8Mb.

- Negative controls showed no signs of contamination through Qubit or Nanodrop

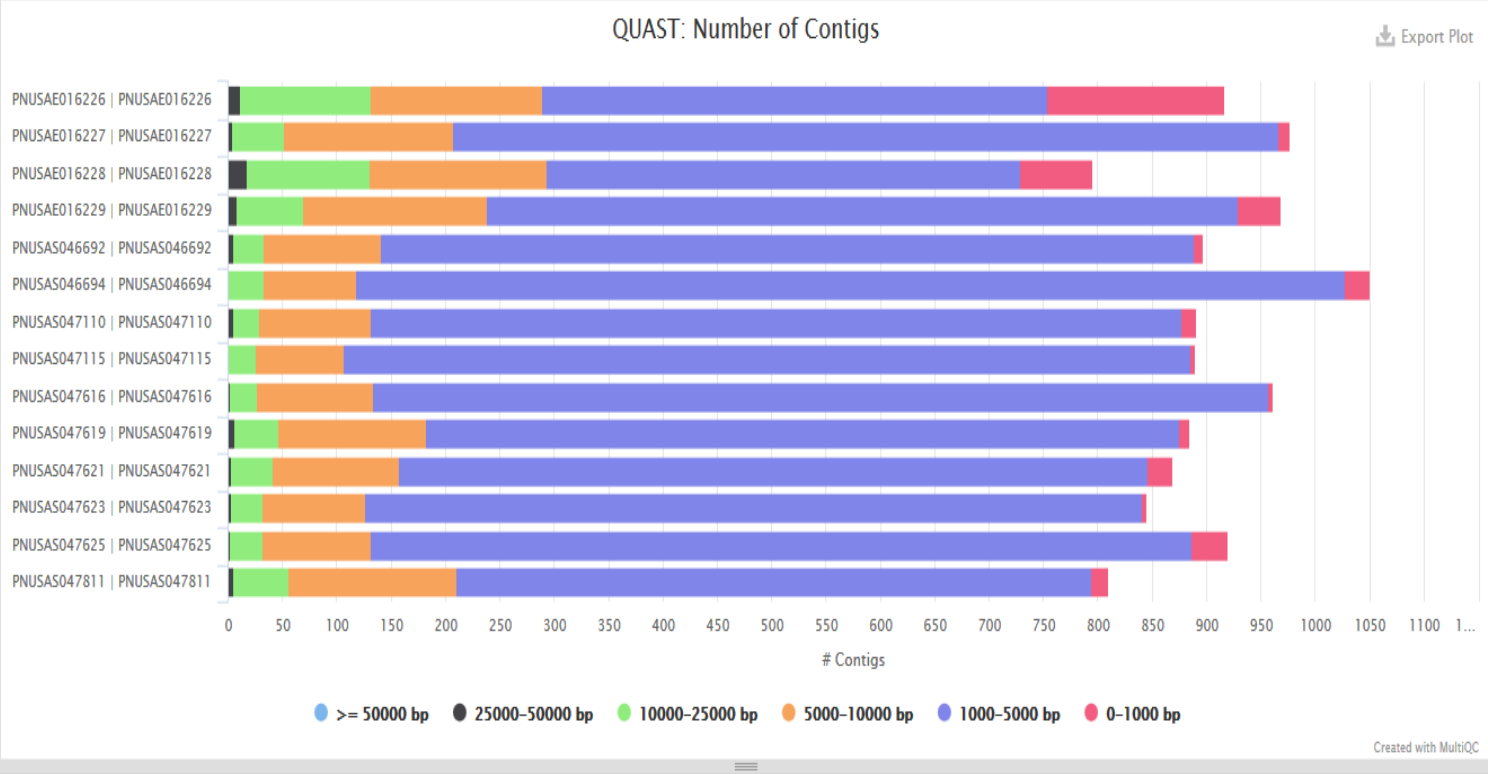
MultiQC
v1.6

- General Stats
- QUAST
  - Assembly Statistics
  - Number of Contigs
- Trimmomatic
- FastQC
  - Sequence Counts
  - Sequence Quality Histograms
  - Per Sequence Quality Scores
  - Per Base Sequence Content
  - Per Sequence GC Content
  - Per Base N Content
  - Sequence Length Distribution
  - Sequence Duplication Levels
  - Overrepresented sequences
  - Adapter Content

## Number of Contigs

This plot shows the number of contigs found for each assembly, broken down by length.

Counts | Percentages



# Problem

- End of July 2018 – early August 2018 we had 8 runs with contaminated specimens.
- All specimens were extracted by MagNA pure LC
- No contamination in hand extracted specimens.

# Troubleshooting

- Wipe tests were performed on the MagNa Pure
  - 16s – positive
  - Stx1 and Stx2 – positive
- Cleaning was performed on the machine and wipe tests were negative.
- A checkerboard extraction was performed
- Water tested with PCR - positive

- More cleaning was performed
  - When cleaning on the inside of the nozzle head where the pipettes are held, some black gunk was found and removed
- Another wipe test and checkerboard extraction was performed – Negative
- MagNa Pure extracted samples were library prepped and ran

# Sources of contamination

- Hypothesis
  - Too much bug was being put into the machine for extraction?
  - Incorrect use of the machine?
    - Wells empty but chosen on the machine?
  - Insufficient decontamination between runs?

# Future Steps

- Negative control taken through library preparation and sequencing
  - Specimens are library prepped and ran with the negative control that they were extracted with
- Implemented deeper cleaning of MagNa Pure on a weekly basis.
- Monthly wipe tests
- Continue to look at more metrics for our specimens to detect anomalies in real time.