

# WHOLE-GENOME SEQUENCING IN DSHS LABORATORY

Laboratory Director's Meeting  
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# OUTLINE



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- WGS in DSHS Laboratory
- How does it work?
- Current status
- Future of WGS at DSHS
- Limitations/Barriers to WGS
- Conclusions

# WGS in DSHS Laboratory



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- Nov. 2014 - selected to join the Genome Trakr network
  - ▣ FDA provides:
    - Loaner instrument
    - Training
    - Reagents & supplies provided
    - Bioinformatics capability
  - ▣ DSHS must sequence 400 bacterial isolates, the majority from food or environmental sources
    - Salmonella, Shigella, Listeria, Campylobacter, E. coli 0157, non-0157 STEC

- CDC ELC Grant
  - ▣ Requested funding for reagents/supplies to sequence an additional 250 clinical outbreak specimens
    - Only received part of the funding from ELC, but receiving supplemental funding from APHL
      - Reagents and training
    - CDC can provide data analysis
  - ▣ APHL to provide BioNumerics 7.5 upgrade to support WGS activities for PulseNet
    - BioNumerics 7.5 is capable of analyzing molecular subtyping information, including PFGE, whole genome sequencing, etc.

# Whole Genome Sequencing



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## Processing Steps:

- ❑ Extract DNA from pure isolates
- ❑ DNA quantification
- ❑ Library prep
  - ▣ Tagmentation
  - ▣ PCR
  - ▣ PCR product cleanup and size selection
  - ▣ Normalization (to make sure each isolates is equally represented)
- ❑ Library pooling
- ❑ Run on machine (40 hr.)

## Data Analysis

- ❑ Stream data to BaseSpace (Amazon Cloud)
- ❑ Do quality checks/sequence analysis
- ❑ Upload raw data to NCBI

# Current Status



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- Staff
  - ▣ 1 fully trained (FDA & CDC) staff who has been certified by CDC to perform and upload sequences
  - ▣ 5 cross-trained staff
    - 1 CDC certified for WGS
    - 1 pending CDC analysis for certification

# WGS Progress



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- Started performing sequencing in late April 2015
  - ▣ 157 clinical isolates
  - ▣ 121 food/environmental isolates
  
- Plan to sequence ~650-800 isolates per grant year
  - ▣ ELC & FDA
  
- Max capacity = 48 samples/week

# Future of WGS at DSHS



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- Very likely that WGS will replace PFGE in next few years (as early as 2 years from now)
- Cost ~ similar
  - WGS estimated at \$100-\$150/isolate (CDC)
  - PFGE – \$112 (DSHS)
  - WGS may provide enough additional information to be cost effective
    - Reflex tests not needed
- Requires upgrade to Bionumerics 7.5 or higher



# Future of WGS at DSHS



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- *Mycobacterium tuberculosis* complex (MTBC) reference labs
  - ▣ Labs being set up to sequence 16-64 MTBC samples per month
- Gonococcal Isolate Surveillance Project (GISP)
  - ▣ Drug susceptibility surveillance on *N. gonorrhoeae* isolates
- 2<sup>nd</sup> tier SCID Screening

# Limitations/Barriers to WGS

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- Data Analysis & Computing Power/Capacity
  - ▣ No bioinformatics software or expertise
  - ▣ Currently utilize CDC & FDA for analysis
  - ▣ NCBI makes phylogenetic trees for Listeria, E. coli, Campylobacter and Salmonella (in collaboration with CDC and Genome Trakr network)

# Limitations/Barriers to WGS

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- Capacity
  - ▣ 1 instrument
  - ▣ Staff have multiple responsibilities
- Method
  - ▣ DNA Extraction – research shows comparable WGS results as long as sequencing data pass QC check
  - ▣ Multiple protocols to follow for data flow

# Conclusions

- Huge amounts of potential
- Limited capacity at this time
  - ▣ Goal – to develop and increase capacity
- Biggest challenge is sequence analysis
  - ▣ Lack software
  - ▣ Lack bioinformatician
  - ▣ Lack computing power

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# ANY QUESTIONS?

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