Food Safety and Inspection Service
Protecting Public Health and Preventing Foodborne Illness
FSIS: WGS for Surveillance and Outbreak Detection

InFORM 2017 Orange Grove, CA
November 6-9, 2017

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Food Safety and Inspection Service:

FSIS: Update on WGS

• Background

• How our data fits with public health mission

• Concluding remarks
Why WGS?

- **Improved resolution for foodborne illness investigations**
  - Improved strain discrimination, illness cluster detection, and case classification

- **Supports FSIS mission goals**
  - Effectively use science to understand foodborne illness and emerging microbiological trends
  - Identification of environmental harborage or recurrences of pathogens in FSIS-regulated establishments/products to further support the inspection and verification process

- **Alignment of pathogen surveillance with our domestic public health and regulatory partners**
  - Collaborative efforts with US Food and Drug Administration Center for Food Safety and Applied Nutrition (FDA-CFSAN), the US Centers for Disease Control and Prevention (CDC), the US National Institutes of Health National Center for Biotechnology Information (NCBI), and also state/local health partners/laboratories
Food Safety and Inspection Service:

FSIS Public Meeting on Whole Genome Sequencing, Oct 26th-27th, 2017

• **Purpose:** To provide a forum to allow public health partners and industry and consumer stakeholders to share information on the use of WGS to improve food safety and public health.

• **Representation:**
  – Federal and State WGS use and strategy updates: CDC, USDA (FSIS), FDA (CFSAN and CVM), NCBI, Mn Dept. of Health.
  – International perspectives: CFIA, SENASICA, GMI
  – Stakeholder perspectives: Cargill, United Fresh Association, private consulting and consumer groups.

• **Discussion points:**
  – Stakeholder concerns included uncertainties on the regulatory use of WGS data
  – International, Federal, and state agencies are involved in collaborative efforts for the harmonization of laboratory and bioinformatic procedures
  – Continued collaboration regarding the use of WGS will be necessary to accomplish Healthy People foodborne illness reduction goals
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**WGS at FSIS: Current Status**

- FSIS has built capacity for conducting WGS on all isolates obtained from FSIS sampling programs
  - Currently 12 sequencers in FSIS Field Service Laboratories, and expect all to be operational in early FY18
  - In FY17, FSIS sequenced 7,282 isolates
- In collaboration with our public health and regulatory partners, FSIS currently considers available WGS analyses in addition to PFGE, epidemiological and trace-back information to further understand the relationship between clinical and food isolates
- FSIS works with National Antimicrobial Resistance Monitoring System (NARMS) partners (FDA, CDC) to understand the occurrence or introduction of antimicrobial resistance genes in pathogens of interest
### FSIS Submissions to NCBI Bioprojects

- **PRJNA242847**
  - GenomeTrakr Project: USDA-FSIS (*Salmonella*)
- **PRJNA215355**
  - GenomeTrakr Project: FDA (*Listeria monocytogenes*)
- **PRJNA287430**
  - USDA-FSIS: *Campylobacter*
- **PRJNA268206**
  - GenomeTrakr Project: USDA-FSIS (STEC)
- **PRJNA292666**
  - FSIS NARMS *Salmonella*
- **PRJNA292667**
  - FSIS NARMS *E. coli*
- **PRJNA292668**
  - FSIS NARMS *Campylobacter*
- **PRJNA292669**
  - FSIS NARMS *Enterococcus*

### Number of Isolates Sequenced by Fiscal Year

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Number of Isolates</th>
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</thead>
<tbody>
<tr>
<td>FY2014</td>
<td>0</td>
</tr>
<tr>
<td>FY2015</td>
<td>2000</td>
</tr>
<tr>
<td>FY2016</td>
<td>3000</td>
</tr>
<tr>
<td>FY2017</td>
<td>7000</td>
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</tbody>
</table>
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WGS Data Sharing – Metadata

- Description about an isolate that has an experiment assigned
- Metadata, such as source type, organism, serotype etc.
- BioSample number is specific to a bacterial isolate for FSIS
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Further Characterization of Isolates Using WGS

- *Campylobacter* speciation
- Serotyping/serogrouping
  - *Salmonella*
  - Adulterant STEC
- Antimicrobial Resistance (Phenotype prediction)
  - *Salmonella*
  - *Campylobacter*
  - *E. coli*
  - *Enterococcus*
- Identify characterized genes of interest
  - Resistance to environmental factors (heat, acid, etc)
  - Virulence factors
    - *stx/eae* sub-types (STEC)
- Alternative to PFGE for comparison of genotypes
  - wgMLST analyses
  - SNP analyses

A single workflow for many characterization approaches via informatics
Food Safety and Inspection Service: FSIS: WGS Data Analyses Work Flow Overview

**Input:** FASTQ

- MLST Sequence Type
- Antibiotic Resistance genes
- Virulence Profile
- Salmonella and STEC serotype
- MASH Tree comparison

**Output:** FASTA

- wgMLST BioNumerics 7.6
- Lyve-SET, SNP Pipeline
- NCBI Pathogen Isolate Browser

**De novo Assembly**

**Input:** FASTA

- QC Pipeline
  - Coverage
  - Average Quality
  - Nucleotide balance

**Output:** FASTA

- QC Pipeline
  - File Size
  - N50 & No. contigs
  - Correct organism
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WGS Analyses for Phylogenetic Context: wgMLST and hqSNP Analyses

FSIS uses pipelines developed by public health partners:

- NCBI Pathogen Detection Isolates Browser


- FDA SNP Pipeline https://github.com/CFSAN-Biostatistics/snp-pipeline

- wgMLST BioNumerics 7.6 CDC-PulseNet
### Food Safety and Inspection Service

**WGS usage: SNP tree from NCBI Pathogen Pipeline**


<table>
<thead>
<tr>
<th>Product</th>
<th>Serotype</th>
<th>Total #</th>
<th>% in SNP cluster (#)</th>
<th>% w/in 20 SNPs of clinical</th>
<th>% w/in 10 SNPs of clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Serotypes</td>
<td>Enteritidis</td>
<td>447</td>
<td>99.8% (446)</td>
<td>98.9% (442)</td>
<td>90.6% (405)</td>
</tr>
<tr>
<td>Kentucky</td>
<td>607</td>
<td>99.8% (606)</td>
<td>0% (0)</td>
<td>0% (0)</td>
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</tr>
<tr>
<td>Heidelberg</td>
<td>74</td>
<td>98.6% (73)</td>
<td>98.6% (73)</td>
<td>86.4% (64)</td>
<td></td>
</tr>
<tr>
<td>Schwarzengrund</td>
<td>122</td>
<td>100% (122)</td>
<td>47.5% (58)</td>
<td>7.3% (9)</td>
<td></td>
</tr>
<tr>
<td>Infantis</td>
<td>218</td>
<td>94.5% (206)</td>
<td>92.2% (201)</td>
<td>50.9% (111)</td>
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<tr>
<td>Typhimurium</td>
<td>155</td>
<td>96.8% (150)</td>
<td>30.3% (47)</td>
<td>10.3% (16)</td>
<td></td>
</tr>
</tbody>
</table>
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WGS usage: SNP tree from NCBI Pathogen Pipeline

Large range of SNPs between PFGE pattern JEGX01.0004 isolates, isolates with different PFGE patterns clustering with pattern JEGX01.0004 isolates.
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WGS Analyses for Phylogenetic Context: wgMLST and hqSNP Analyses

- FSIS Pork 2016 Isolate
- Clinical 2016
- Clinical 2015
- Unrelated (No epi)

Settings: Reference 2013k-0676 obtained from CDC-EDLB (PacBio assembly) had no phage masking (phage masking is not done for i:4:5:12:i:- routinely). The analysis was generated with Lyve-SET version 1.1.4f. Reads were cleaned with CG Pipeline, SNPs were called with VarScan, and Lyve-SET was run with the following options: minimum coverage- 10, min alternative fraction- 0.90, and allowed flanking- 5 bp. Maximum Likelihood Tree built in Mega Version 7 with 500 bootstraps.
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WGS Analyses for Phylogenetic Context: wgMLST and hqSNP Analyses

BioNumerics version 7.6
- wgMLST scheme for *Lm*
- *Lm* Pilot Lab Participant
- Six analysts with CDC Certification completed
- High quality data uploaded to CDC National Database
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WGS usage: hqSNP analyses and wgMLST with Listeria monocytogenes (case of potential harborage)

Agreement between three pipelines
FSIS’ new strategic plan is focused on the use of new technology to prevent foodborne illnesses and protect public health
  – Includes two objectives specific to incorporating WGS into FSIS surveillance and regulatory efforts

FSIS has built sufficient capacity for conducting WGS on all FSIS pathogen isolates
  – Sequenced 7282 isolates in FY17 with the future target of ~10,000 isolates/year

FSIS is committed to using WGS data to support outbreak investigations

FSIS continues to engage with all national and international partners

FSIS continues to use WGS analyses in conjunction with other metadata, including epidemiological and trace-back information
Food Safety and Inspection Service:

Acknowledgements

- USDA FSIS Offices
- USDA ARS
- CDC PulseNet and NARMS
- FDA CFSAN
- FDA CVM
- NCBI
- State Laboratories