



United States Department of Agriculture

# OneUSDA “Do right and feed everyone”



## Food Safety and Inspection Service

Protecting Public Health and Preventing Foodborne Illness



Food Safety and Inspection Service:

# **Managing the Transition to WGS and Maintaining Multiple Workflows in USDA FSIS**

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## Food Safety and Inspection Service:

### Why WGS?

- Improved resolution for foodborne illness investigations
- Supports FSIS mission goals
  - Understand foodborne illness and emerging microbiological trends
  - 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



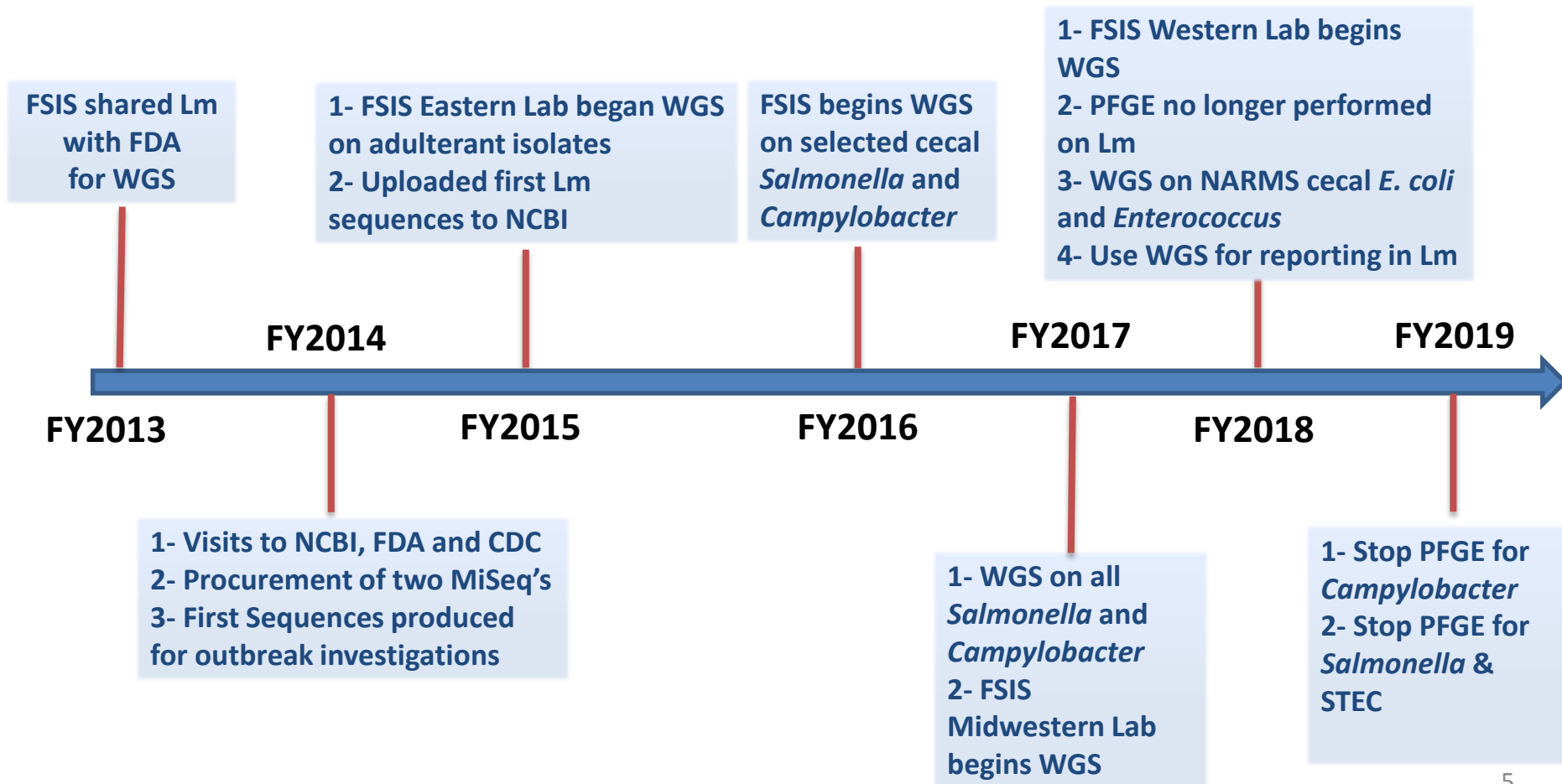
## Food Safety and Inspection Service:

### WGS at FSIS: Current Status

- Performs WGS on all isolates from FSIS sampling programs
  - 12 sequencers in FSIS Field Service Laboratories
  - In FY18, FSIS has sequenced ~11,000 isolates
- Use WGS analyses in addition to epidemiological and traceback information to further understand the relationship between isolates
- Work with National Antimicrobial Resistance Monitoring System (NARMS) partners (FDA, CDC) to understand the occurrence or introduction of antimicrobial resistance genes in pathogens of interest

# Food Safety and Inspection Service

## WGS at FSIS: Capability and Capacity Building by Year



# Food Safety and Inspection Service: WGS Data Sharing – Metadata and Sequence Data

https://www.ncbi.nlm.nih.gov/biosample/?term=FSIS1606985

NCBI Resources How To usda fsis oi My NCBI Sign Out

BioSample BioSample FSIS1606985 Search

Full Pathogen: environmental/food/other sample from *Listeria monocytogenes*

Identifiers BioSample: SAMN05366775; Sample name: FSIS1606985; SRA: SRS1548159

Organism [Listeria monocytogenes](#)  
cellular organisms, Bacteria, Terrabacteria group, Firmicutes, Bacilli, Bacillales, Listeriaceae, Listeria

Package [Pathogen: environmental/food/other, version 1.0](#)

Attributes

strain	FSIS1606985
collected by	USDA-FSIS
collection date	2016
geographic location	<a href="#">USA WV</a>
isolation source	Product-Swab-Non meat
latitude and longitude	missing

BioProject [PRJNA215355](#) *Listeria monocytogenes*  
Retrieve [all samples](#) from this project

Submission USDA FSIS, Glenn Tillman, 2016-07-08

Accession SAMN05366775 ID: 5366775  
[BioProject](#) [SRA](#)

Send to: Related information BioProject SRA Taxonomy

Search details FSIS1606985 [All Fields] Search See more

Recent activity Turn Off Clear

- Pathogen: environmental/food/other sample from *Listeria monocytogenes* biosample
- FSIS1606985 (1) BioSample
- Enterococcus BioProject
- PRJNA292668 (1) BioProject

## 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*

- 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

# Food Safety and Inspection Service: FSIS: WGS Data Analyses Work Flow Overview

## De novo Assembly



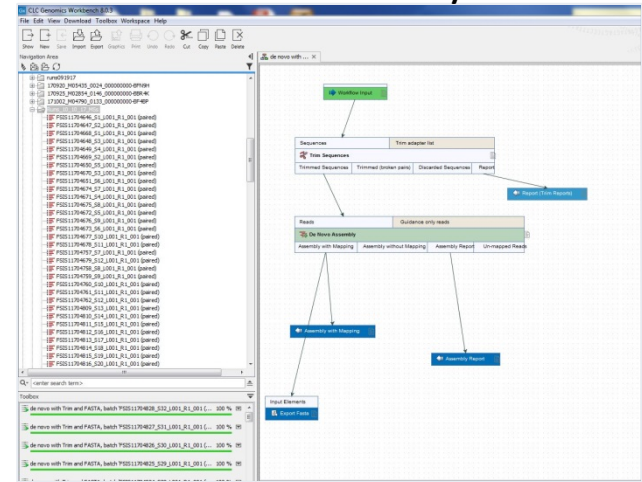
Input: FASTQ

QC Pipeline

- Coverage
- Average Quality
- Nucleotide balance

```
@M02848:54:000000000-AJ347:1:1101:14587:1926 1:N:0:4
TCCGTGCTCAGTTACACGGACAAAATACGGCGAAAAACCTTGGCCCTCCCTGGCGACATGGGATT
+
AA>1A1AAD31DF3F3B11A0000B1BB00A/////0AAGG11/BFFGFFFG1/>//E/210E0FGDGF1
```

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



Output: FASTA

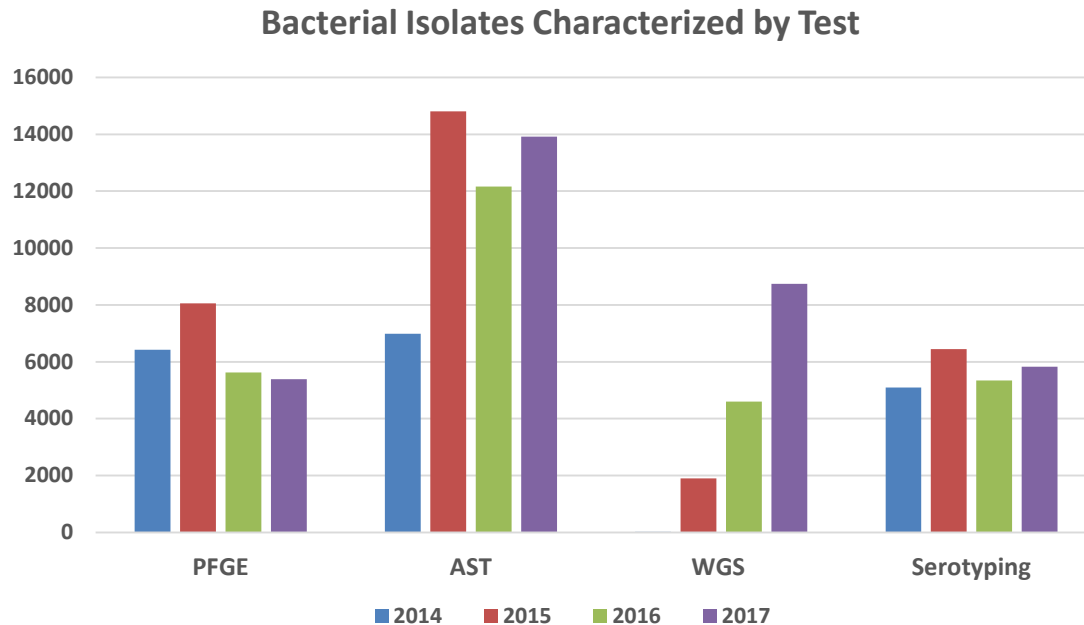
- QC Pipeline
- File Size
  - N50 & No. contigs
  - Correct organism

Input: FASTA

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

```
>FSIS1609314_s10_L001_R1_001_(paired)_trimmed_(paired)_contig_1
TGCGGTTTTGTACGTTCAAATTTTTCTTTAGACACGGCTATATCTTACTATAAGCGCTC
TCCCCTTCAGGAGAGAGCACGGGATTTGGTTTTTAACCCCTGCGGCTTATTTACCACGGG
CTTCGATTACGGCCTGAGCAACGTTGTTTCGGCGCATCATCACTTCAGGAATTCATAG
TGTACGATGCGCGACCTTTGGTCAGAGAACGCGAGCTGAGTTCAGATCCGGAACATTCAG
ACAGCGGTACTTCAGCGGGAATCTTAACGCTGTAACCTTCAGATCCTGACCTTTGAGCA
TACCACGACGGCGGCTAAGGTCGCCGATAACGTCACCGGATTTCTTTCAGGTGTTCTA
CTTCAACCTTCATGATCGGCTCAAGCAGAAGCTGTTTTGCTTCTTAAAGCCTTCTTTAA
AGCGGATAGACGACGGCAGTTTAAACGCCAGCTCAGAGGAGTCAACGTCATGGTAAGAAC
```

# Food Safety and Inspection Service: Maintaining Parallel Workflows



- Four main characterization tests currently performed in parallel



# Food Safety and Inspection Service:

## 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, sanitizers, 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:

## WGS Analyses for Phylogenetic Context: wgMLST and hgSNP Analyses

FSIS uses pipelines developed by public health partners

Lyve-SET

<https://github.com/lskatz/lyve-SET/blob/master/>

NCBI Pathogen Detection Isolates Browser

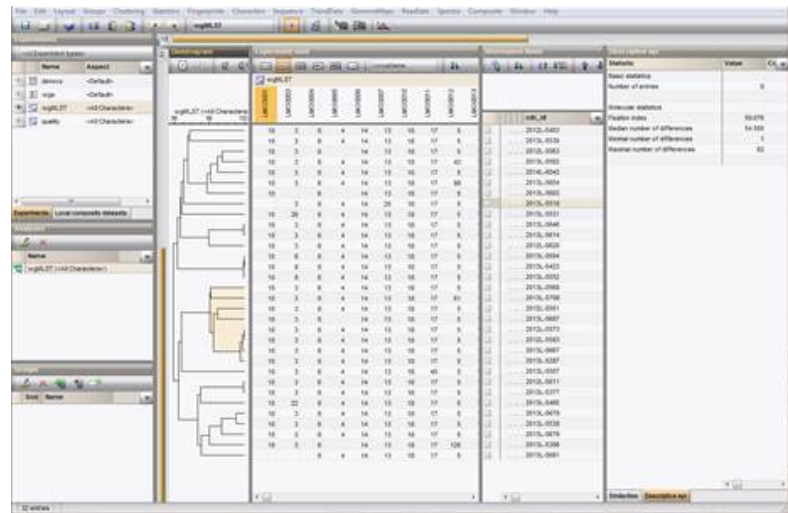
<http://www.ncbi.nlm.nih.gov/pathogens>

FDA SNP Pipeline

<https://github.com/CFSAN-Biostatistics/snp-pipeline>

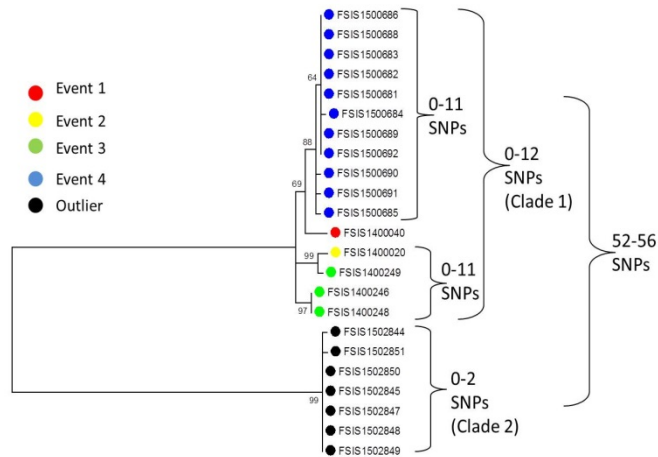
wgMLST

BioNumerics 7.6 CDC-PulseNet

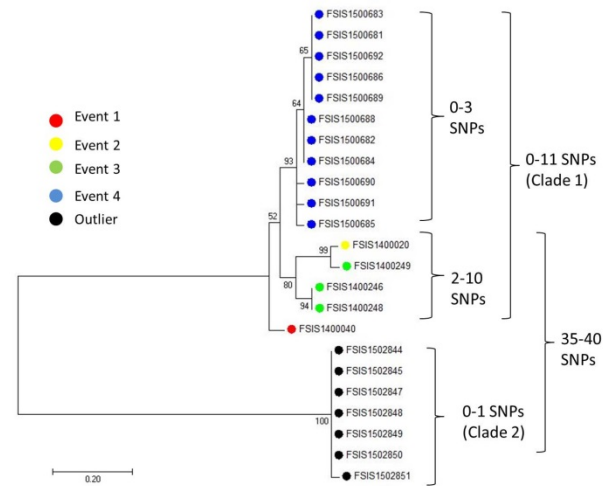


# Food Safety and Inspection Service

## WGS usage: **hqSNP** analyses and **wgMLST** with *Listeria monocytogenes*

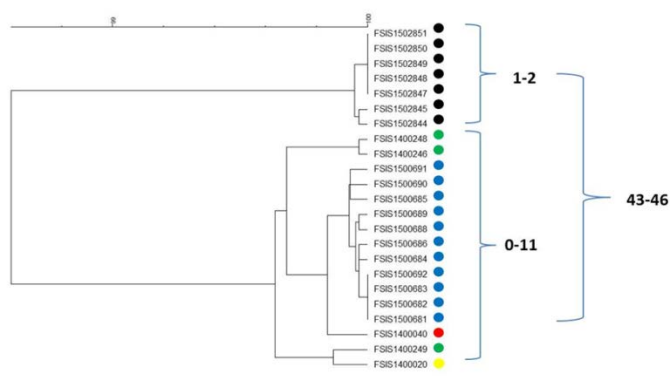


**Snp Pipeline**



**Lyve-SET**

wgMLST (All Characters)



**wgMLST**

- Est. A Sampling Event 1
- Est. A Sampling Event 2
- Est. A Sampling Event 3
- Est. A Sampling Event 4
- Unrelated Est. Sampling Event



**NCBI Pathogen Browser hqSNP**

## Food Safety and Inspection Service:

### Concluding Remarks

- High-throughput capacity for WGS
  - Sequenced ~11,000 isolates in FY18 to date
- FSIS has performed parallel workflows for several years
- FSIS labs will utilize WGS data in outbreak investigations, and as a stream-lined analytical procedure

# Food Safety and Inspection Service: **Acknowledgements**

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

