



GenomeTrakr database and network: WGS network for real-time characterization and source tracking of foodborne pathogens

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Almond Board Food Quality and Safety Symposium: June 2019

(NSF.)

NGS Costs

Cost per Raw Megabase of DNA Sequence



genome.gov/sequencingcosts



Illumina Suite

- > Massively parallel, short read, Sequencing-by-Synthesis
- > Cluster generation, reversible dye-terminator dNTPs
- Instruments range from low to high throughput:
 iSeq, MiniSeq, MiSeq, NextSeq, HiSeq, NovaSeq
- Read Length:
 Paired end reads (forward and reverse)
 150-300 bp long (300-600 total)
- > Max Output: 4M to 20B Reads

From 1.2 GB to 6 TB





Burnet

Ion Torrent by ThermoFisher Scientific

- > Ion Torrent semiconductor sequencing
- > Measures hydrogen ions (pH) during sequencing-by-synthesis reactions
- > No modified bases required

NSF

- > Instruments: Ion S5, S5 Plus, S5 Prime
 - 200-600 bp read length
 - Up to 130M Reads
 - Scalable output from 15 to 25 GB



https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-run-sequence/ion-s5-ngs-targeted-sequencing.html



SMRT Sequencing by PacBio

- Single Molecular, Real-Time (SMRT)
 Sequencing
- > Zero-mode waveguides and phospholinked nucleotides (dyes)
- > PacBio RS II Original Long Read Sequencer
 - 60,000 bp max read length
 - 0.5-1 Gb per cell
 - Up to 16 cells per run
- > PacBio Sequel High throughput WGS
 - Targeting *de novo* assemblies
 - ~365,000 reads per cell
 - 5-8 Gb per cell



http://www.pacb.com/products-and-services/pacbio-systems/

Oxford Nanopore Technologies

- > Single molecule, real-time, nanopore sequencing
- > MinION Portable Sequencer
 - 5-10 Gb per flow cell
 - Extreme read lengths (record is > 2 MB)
 - Real-time data analysis
 - Compact size.
 Weighs <100g and plugs into PC via USB
- > PromethION High-throughput Sequencer
 - Up to 48 flowcells
 - Simultaneous sample processing
 - Increases data yield to TB
- > SmidgION smartphone device in development







Why Develop a WGS based Network?

- Tracking and Tracing of food pathogens
 - Insufficient resolution of current tools

 matching clinical to environmental
 improve the environmental database
 - Faster identification of the food involved in the outbreak
 - Limited number of investigators vs. facilities and import lines
 - Global travel
 - Global food supply





Benefits of a WGS Approach

- More discriminatory and informative than PFGE
- Clues to geographic origin of pathogen

This means:

- Greater certainty when matching clinical, environmental, and product sample isolates
- Links between illnesses and the potential source of contamination can be made with fewer isolates
- Investigators can be deployed in a more targeted manner, saving resources

End Result:

- Faster identification of the food involved in the outbreak
- Potential to help reduce the number of foodborne illnesses and deaths over the long term both in the U.S. and abroad.



Current Scope of GenomeTrakr Network

- Network includes labs at FDA, CDC, FSIS, 17 state health and university labs, 1 U.S. hospital lab, and 11 labs located outside the U.S.
 - Contributing labs are on 4 continents and in 10 countries
- The network provides high resolution genomic sequences of food pathogens, ex. *Salmonella, Listeria*, STEC's, others. Greater than 250,000 sequences in the database
- New GenomeTrakr labs are coming on-line
- Partnered with CDC in 2013 to study all clinical and environmental isolates of Listeria monocytogenes, now E. coli, (Salmonella coming)

FDA GenomeTrakr website http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ucm363134.htm



U.S. GenomeTrakr Labs





Labs Outside the U.S. Contributing to GenomeTrakr





Basic Data Flow for Global WGS Public Access Databases

DATA ACOUISITION

Sequence and upload genomic and geographic data



State, Local, Federal, and Foreign Public Health Agencies

Academia/Industry



Total Number of Sequences in the GenomeTrakr Database

Number of Sequences (as of the last day of the quarter)

May 09, 2019 GenomeTrakr Numbers



Species	Total Isolates
Salmonella enterica	196 325
Sumonena enteriea	190,923
E.coli and Shigella	71,760
Campylobacter jejuni	29,770
Listeria monocytogenes	26,923
Vibrio parahaemolyticus	1,859
Cronobacter	605
Total	327,242



How do we use the GenomeTrakr information? Example of Listeria in sprouts using a phylogenetic perspective.







Further perspectives on the food supply



CENTER FOR FOOD SAFETY & APPLIED NUTRITION

Once tomatoes reach the supply chain, things really "simplify".



The Fresh-cut Tomato Supply Chain





Scenario 2 and statistics: Resident Contamination

Field 1





Post inspection determines root cause of contamination event.







Whole Genome Tree



Key Conclusions from VA surveys Identify Water, Water, and Water

- Sampled Areas:
 - Virginia Tech Agricultural Research and Education Center (AREC):
 2009-2011
 - 6 environmental waters and sediments: 2011, 2012, 2014-2015
- *Salmonella* Pattern 61 and other clinically relevant isolates found consistently in waters and sediments at AREC and the 6 other locations
- Environmental waters and sediments are potential reservoirs for Salmonella
- Enhanced agricultural practices related to the use of surface waters are important to mitigating *Salmonella* transfer on to crop plants



Ecological prevalence, genetic diversity, and epidemiological aspects of Salmonella isolated from tomato agricultural regions of the Virginia Eastern Shore. Bell RL et al







Current Status

- WGS is now routine in FDA's outbreak response and compliance/surveillance. Internally (across our agency), and in collaboration with FSIS and CDC, WGS has now been deployed and benefitted the traceability of numerous foodborne contamination events. Weekly Regulatory Guidance from SME.
- Numerous offshoot applications exist (i.e., supply chain management, quality assurance, process evaluation, etc.).
- Genome sequences are **portable**, **instantly cross-compatible and highly scalable**. One technology approach irrelevant of organism.
- Have to balance the need for increased number of well characterized **environmental** (food, water, facility, etc.) sequences with the need for extensive clinical isolates
- WGS, unlike PFGE, is more than a surveillance tool. It provides information on **AMR**, Virulence, serotype, and other critical factors in one assay, including historical reference to pathogen emergence.
- The volume of WGS and associated work continues to grow. The CDC currently sequences all ~800
 Listeria clinical samples they receive annually, and we expect them to start sequencing all STECs and Salmonella clinical samples by 2017.
- As internal and external sequence data is integrated and analyzed in concert, we expect many more Illnesses that were previously attributed to **sporadic infections** to now be linked to specific food or environmental sources. This will result in the detection of a **large number of small outbreaks**.

www.ncbi.nlm.nih.gov/pathogens/

S Home - Pathogen Detect X

C https://www.ncbi.nlm.nih.gov/pathogens/

Pathogen Detection **BETA**

NCBI Pathogen Detection integrates bacterial pathogen genomic sequences originating in food, environmental sources, and patients. It quickly clusters and identifies related sequences to uncover potential food contamination sources, helping public health scientists investigate foodborne disease outbreaks.

Find isolates now!

Explore the Data

Species	New Isolates	Total Isolates
Salmonella enterica	<u>124</u>	<u>53,003</u>
E.coli and Shigella	1	<u>19,861</u>
Listeria monocytogenes	<u>20</u>	12,267
<u>Campylobacter jejuni</u>	0	<u>4,309</u>
Acinetobacter baumannii	0	<u>2,651</u>
<u>Klebsiella pneumoniae</u>	1	2,212
Enterobacter	0	<u>1,151</u>
<u>Vibrio parahaemolyticus</u>	0	<u>817</u>

Learn More

<u>About</u>

<u>FAQ</u>

Antimicrobial Resistance

FDA U.S. FOOD & DRUG

Contributors

Data Resources

Isolates Browser

Antimicrobial resistance reference gene database

Isolates with antibiotic resistant phenotypes

Beta-lactamase resources

Download analysis results (FTP)

Submit

How to submit data

How to submit antibiotic resistance phenotypes

How to submit beta-lactamases

NCBI Submission Portal



New Isolate Check - Listeria

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NCBI National Center for Biotechnology Information

Health > Pathogen Detection

Pathogen Detection **BETA**

NCBI Pathogen Detection integrates bacterial pathogen genomic sequences originating in food, environmental sources, and patients. It quickly clusters and identifies related sequences to uncover potential food contamination sources, helping public health scientists investigate foodborne disease outbreaks.

Find isolates now!

Explore the Data

Species	New Isolates	T otal Isolates
Salmonella enterica	<u>368</u>	<u>63,000</u>
E.coli and Shigella	1	26,603
Listeria monocytogenes	<u>3</u>	13,343
<u>Campylobacter jejuni</u>	<u>11</u>	<u>7.857</u>
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Learn More

<u>About</u>

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Data Resources

Isolates Browser

Antimicrobial resistance reference gene database

Isolates with antibiotic resistant phenotypes

Beta-lactamase resources

Download analysis results (FTP)



New Isolate Check - Listeria

Health > Pathogen Detection > Isolates Browser

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1 2	Listeria monocytogenes	PNUSAL00		PDT000195504.1	2017-03-21	USA		clinical		PDS000011887.1	n/a	21	SAMN06624016		PDG00000001.646

- 3 New Clinical Isolates
- Inconclusive food/env matches, 21-24 SNPs
- Clinical match, 3 SNPs

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1	SNP cluster	Min-sar	Min-dif	Bi
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	PDS000011887.1	n/a	21	SAN
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PDS000003244.7 Tree View

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2017K–0710 missing USA missing	
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CFSAN024173 2014–07 USA:VA water (10 gal)	
CFSAN024174 2014–07 USA:VA water (10 gal)	\downarrow
CFSAN024176 2014–07 USA:VA water (10 gal)	·
fnw19J13 2014–02–25 USA:OR environmental sample 839242 82–1	3
2014K–0614 missing USA Missing	
2014K-0290 2014-03 USA Urine	
fnw19J11 2014-02-25 USA:OR environmental sample 839242 54-	1
CFSAN058066 2014 USA: WA Environmental Sponge	
-2014K-0603 missing USA Missing	
CFSAN058065 2014 USA: WA Environmental Sponge	
2014K–0606 missing USA Missing	
2014K–0314 missing USA Missing	
CFSAN058068 2014 USA: WA Environmental Sponge	
2014K-0675 2014-01 USA Stool	
CFSAN033398 2014–11–18 USA:WA environmental sponge	
CFSAN058078 2014 USA:WA Environmental Sponge	0 5 SNIP
CFSAN058070 2014 USA: WA Environmental Sponge	0-55815
CFSAN058069 2014 USA: WA Environmental Sponge	
fnw19J12 2014–02–25 USA:OR environmental sample 839242 76–7	
CFSAN058067 2014 USA: WA Environmental Sponge	
CFSAN033399 2014–11–18 USA:WA environmental sponge	
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-CFSAN058076 2014 USA:WA Environmental Sponge	
CFSAN058074 2014 USA:WA Environmental Sponge	
CFSAN058071 2014 USA:WA Environmental Sponge	
CFSAN058075 2014 USA:WA Environmental Sponge	
CFSAN058073 2014 USA:WA Environmental Sponge	
CFSAN058077 2014 USA:WA Environmental Sponge	



Phylogenetic results of Salmonella enterica clustering pine nuts and clinical isolates, NCBI Pathogen detection cluster PDS000032222.4. The number line corresponds to SNPs, where the branch length is proportional to the number of SNPs present.

510

environmental/other, 2015-10-16, USA:MD, Bulk Pine Nuts, MDA113268, PDT000087115.2
clinical, 2014-05-29, USA, feces, ILBSalm5410230, PDT000031073.2
environmental/other, 2018-08-08, USA, Pesto, 2011K-1668, PDT000360119.1
environmental/other, 2015-02-10, USA: VA, Pine nuts, turkish pine nuts, VA_WGS-00196, PDT000029724.3
o clinical, 2018-03-31, United Kingdom: United Kingdom, human, 286772, PDT000301702.2
environmental/other, 2015-02-10, Turkey, pine nuts, FMA0193, PDT000032606.3
environmental/other, 2015-02-11, USA: NY, Monroe, PESTO, NY_IDR1100031092-9T1, PDT000002725.3
environmental/other, 2014-11-19, USA: NY, Stool, PDT000044058.2
environmental/other, 2015-10-16, USA:MD, Bulk Pine Nuts, MDA113267, PDT000087114.2
environmental/other, 2015-10-16, USA:MD, Bulk Pine Nuts, MDA113269, PDT000087113.2
clinical, 2018-08-08, USA, 2011K-1666, PDT000360216.1



Phylogenetic results of Salmonella enterica clustering hazelnuts and clinical isolates, NCBI Pathogen detection cluster PDS000027740.2. The number line corresponds to SNPs, where the branch length is proportional to the number of SNPs present.

5 10 15

environmental/other, 2017-02-03, USA:OR, in-shell nuts, CFSAN059748, PDT000184883.2 environmental/other, 2017-02-14, USA:OR, in-shell nuts, CFSAN059115, PDT000187063.2 O clinical, 2017-11-28, USA, stool, PNUSAS026569, PDT000267611.2 environmental/other, 2017-02-14, USA:OR, in-shell nuts, CFSAN059114, PDT000187138.2 environmental/other, 2017-02-14, USA:OR, hazelnuts, CFSAN059103, PDT000186833.2 clinical, 2016-11-29, USA, stool, PNUSAS005432, PDT000163497.2 environmental/other, 2017-02-14, USA:OR, hazelnuts, CFSAN059104, PDT000186827.2 clinical, 2017-01-19, USA, 2017K-0016, PDT000179209.2 clinical, 2017-01-19, USA, 2017K-0012, PDT000179221.2 clinical, 2017-01-19, USA, 2017K-0015, PDT000179213.2 environmental/other, 2017-02-03, USA:OR, in-shell nuts, CFSAN059747, PDT000184887.2 environmental/other, 2017-02-03, USA:OR, hazInuts farm shelled, CFSAN059746, PDT000184888.2 environmental/other, 2017-02-03, USA:OR, hazInuts farm shelled, CFSAN059745, PDT000184889.2 environmental/other, 2017-02-03, USA:OR, hazInuts farm shelled, CFSAN059744, PDT000184890.2 clinical, 2018-02-07, USA, PNUSAS033094, PDT000285544.2 Clinical. 2018-02-05. USA. PNUSAS033095. PDT000284989.2 O clinical, 2018-03-09, USA, PNUSAS034629, PDT000293813.2 O clinical, 2017-01-19, USA, PNUSAS007351, PDT000179224.2 O clinical, 2018-02-21, USA, PNUSAS033097, PDT000288349.2

Phylogenetic results of Salmonella enterica clustering nuts and clinical isolates, NCBI Pathogen detection cluster PDS000005469.11. The number line corresponds to SNPs, where the branch length is proportional to the number of SNPs present.

O environmental/other, 2016-11-16, M134, PDT000160717.2
 O environmental/other, 2016-11-16, M135, PDT000160722.2
 O environmental/other, 2019-01-27, United Kingdom: United Kingdom, other, 647809, PDT000452965.1
 O environmental/other, 2017-02-09, United Kingdom: London, Other, 66441, PDT000052993.2
 O environmental/other, 2017-01-30, USA:AZ, Fertilizer, CFSAN073569, PDT000274748.2
 O environmental/other, 2017-01-30, USA:AZ, Fertilizer, CFSAN059241, PDT000183082.2
 O environmental/other, 2017-02-03, USA:CA, Bone Meal Fertilizer, CFSAN059284, PDT000184863.2
 O environmental/other, 2019-02-22, not collected, sg_8326, PDT000468974.1
 O environmental/other, 2017-03-22, not collected, culture, BCW_3057, PDT000197211.1

Phylogenetic results of Salmonella enterica clustering Pistachio and clinical isolates, NCBI Pathogen detection cluster PDS000027237.52. The number line corresponds to SNPs, where the branch length is proportional to the number of SNPs present.

2 4

environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN075603, PDT000294817.2 environmental/other, 2018-02-26, USA:WA, Pistachios, CFSAN075630, PDT000289623.2 environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN076748, PDT000294882.2 o environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN075637, PDT000294873.2 environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN075638, PDT000294865.2 environmental/other, 2016-03-18, USA:CA, Pistachio, CDPHFDLB-F1602021-005A, PDT000115361.2 environmental/other, 2016-03-18, USA:CA, Pistachio, CDPHFDLB-F1602021-005B. PDT000115360.2 environmental/other, 2017-09-27, USA:CA, Environmental sponge, PDT000244761.2 environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088277, PDT000413809.1 environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088276, PDT000413685.1 • environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088283, PDT000413770.1 O environmental/other, 2018-04-09, USA:CA, Environmental sponge, CFSAN077990, PDT000304837.2 O environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN076741, PDT000294822.2 • environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088278, PDT000413807.1 O clinical, 2016-03-24, USA, Urine, PNUSAS001723, PDT000120800.2 O environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088282, PDT000413731.1 –O environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088275, PDT000413674.1 environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN076742, PDT0002948. environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN076749, PDT000294884.2 environmental/other, 2018-03-16, USA:CA, Pistachios, CFSAN076750, PDT000294883.2 environmental/other, 2018-12-03, USA:CA, Nuts, CFSAN088279, PDT000413729.1

Combining Genetic Distances and FOA Facility Information





Yu Wang, James B. Pettengill, Arthur Pightling, Ruth Timme, Marc Allard, Errol Strain, and Hugh Rand (2018) Genetic Diversity of Salmonella and Listeria Isolates from Food Facilities. Journal of Food Protection: December 2018, Vol. 81, No. 12, pp. 2082-2089.



GenomeGraphR: WGS data integration, analysis, and visualization for risk assessment and management: <u>https://fda-riskmodels.foodrisk.org/genomegraphr/</u> Moez Sanaa, Régis Pouillot, Francisco J Garces-Vega, Errol Strain, Jane M Van Doren doi: https://doi.org/10.1101/495309 2018.





From WGS to Antibiotic Resistance Genotype







There are 11 records with 2 flagged genes uploaded in the last 30 days



Economic Analysis of Salmonella outbreaks reduction using WGS

	Canada	United States
Incidence of illness	47,028	1,200,000
Costs to adopt WGS	\$158,340,000	\$100,000,000
QUALY lost	469.75	16,782
Total Illness costs	\$287,770,000	\$3,300,000,000
Total net benefit of adopting WGS	\$90,250,000	\$1,000,000,000

Model assumes 70% reduction in numbers of illnesses due to WGS implementation. Benefits gained due to earlier detection and decreased time to recall food items. United States estimates are adjusted based on increase population size. Additional analysis is needed to adjust to US illnesses and US health care costs.

Monetary Loss from Bacteria Foodborne Illness Total \$13,279,603,000

FD/





Acknowledgements

• FDA

- Center for Food Safety and Applied Nutrition
- Center for Veterinary Medicine
- Office of Regulatory Affairs
- National Institutes of Health
 - National Center for Biotechnology Information

State Health and University Labs

- Alaska
- Arizona
- California
- Florida
- Hawaii
- Maryland
- Minnesota
- New Mexico
- New York
- South Dakota
- Texas
- Virginia
- Washington

- USDA/FSIS and ARS
- CDC
 - Enteric Diseases Laboratory
- INEI-ANLIS "Carolos Malbran Institute," Argentina
- Centre for Food Safety, University College Dublin, Ireland and Irish FSA
- Melbourne (FSA). Australia
- Public Health England, UK
- Institute for Food Safety and Health (IFSH)
- WHO and FAO
- Illumina
- Pac Bio
- Other independent collaborators



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