



**DEIBEL**  
LABORATORIES



## **Whole Genome Sequencing: Applications in Food Safety**

Tom Donohue, Deibel Laboratories



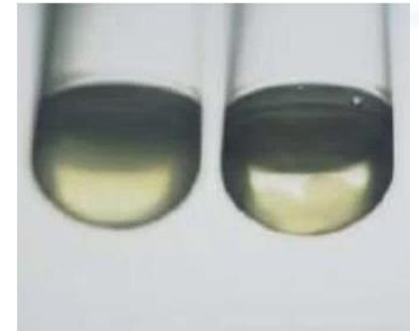
# What is WGS?

- Whole Genome Sequencing
- A technology that provides a nearly complete genetic characterization of a bacterial isolate
  - Identification
  - Subtyping
  - Virulence markers
  - Antimicrobial resistance
- Vast improvement over previous characterization methods
  - Increased resolution
  - Enhanced source tracking
- Powerful tool to aid in outbreak investigations
- Only as good as the database available

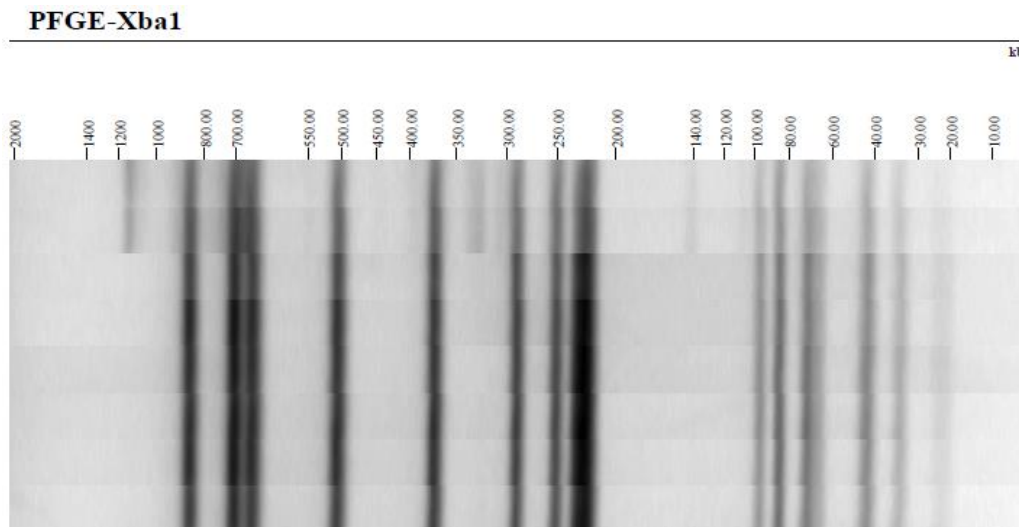


# What preceded WGS?

- Phenotypic subtyping (i.e. serology)
  - *Salmonella* Typhimurium B: i; 1,2
  - *Salmonella* Madison L: d; z<sub>6</sub>



- Molecular subtyping such as Pulsed-Field Gel Electrophoresis or PFGE





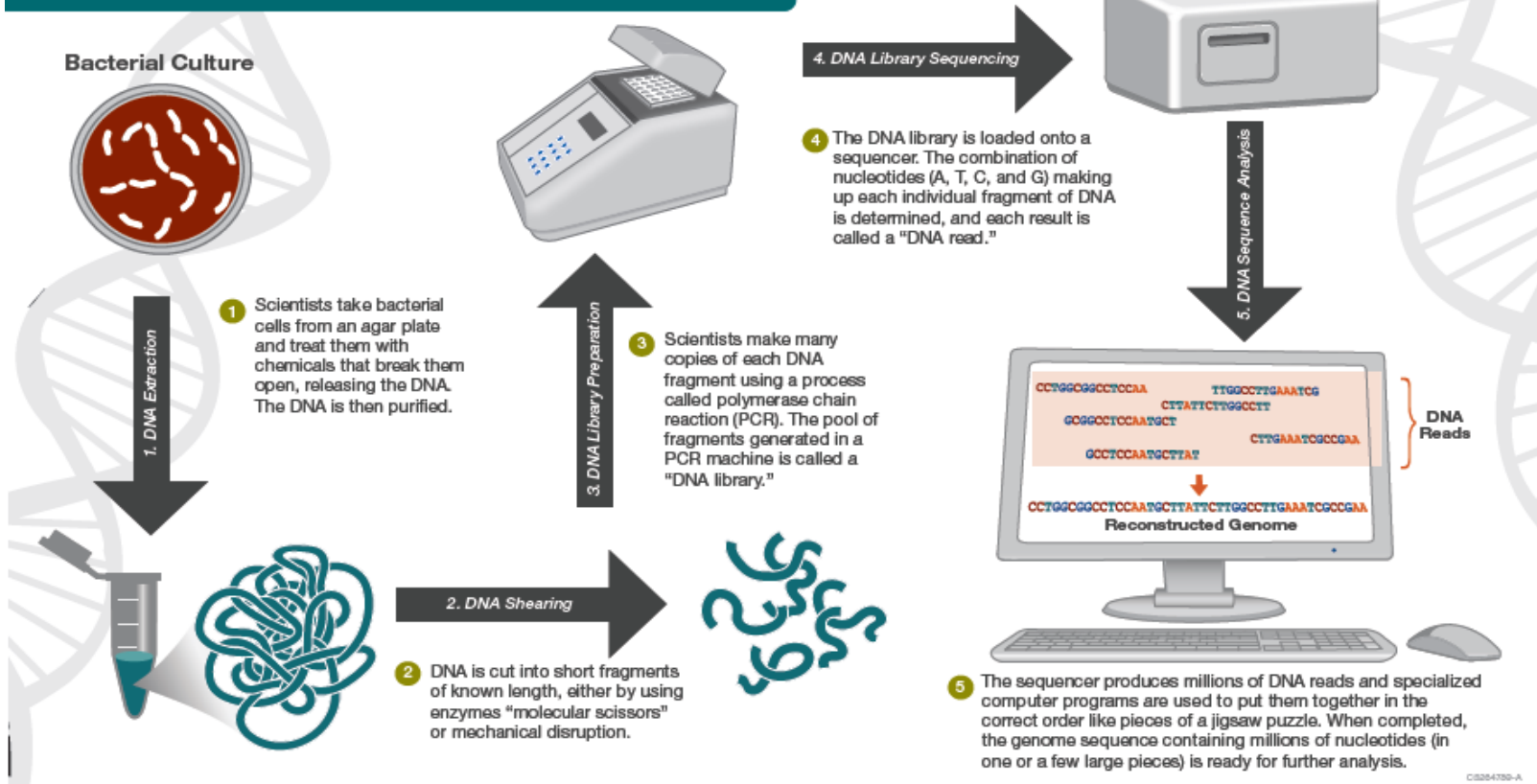
# Requirements for a WGS Testing Program

- Good Laboratory Practices and competent analysts
- Harmonized and accredited methods
- Method validation
- Proficiency testing
- Metadata generation and standardization
- IT solutions and data storage
- Standardized reporting and interpretation of results



## The Whole Genome Sequencing (WGS) Process

WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.



<https://www.cdc.gov/pulsenet/pdf/Genome-Sequencing-508c.pdf>



# Basic Data Flow for Global WGS Public Access Databases

## DATA ACQUISITION

Sequence and upload genomic and geographic data



Other distributed sequencing networks



## DATA ASSEMBLY, ANALYSIS, AND STORAGE

International Nucleotide Sequence Database Collaboration (INSDC)  
Shared Public Access Databases

- NCBI – National Center for Biotechnology Information
- EMBL – European Molecular Biology Laboratory
- DDBJ – DNA Databank of Japan



## PUBLIC HEALTH APPLICATION AND INTERPRETATION OF DATA

- Find clinical links
- Identify clusters
- Conduct traceback
- Develop rapid methods
- Develop culture independent tests
- Develop new analytical software



11/2014

State, Local, Federal, and Foreign Public Health Agencies

Academia/Industry

<https://www.fda.gov/food/whole-genome-sequencing-wgs-program/genometrakr-network>





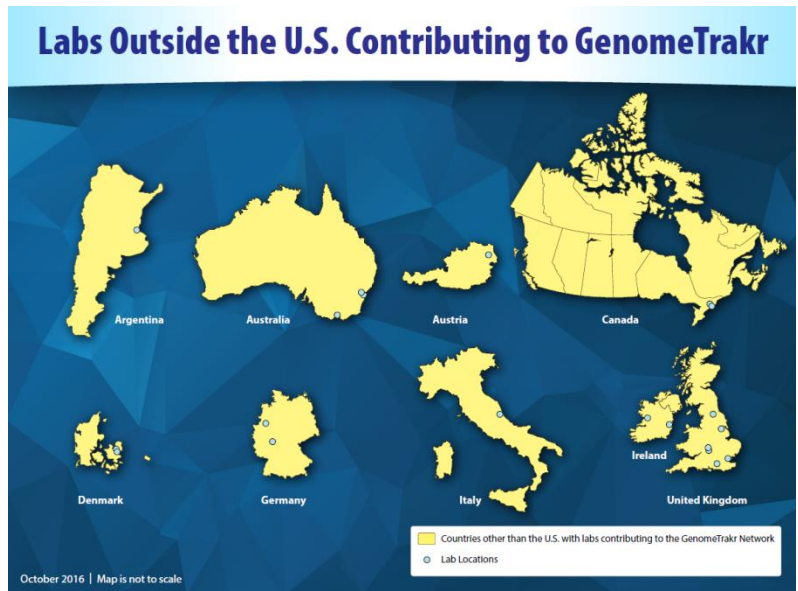
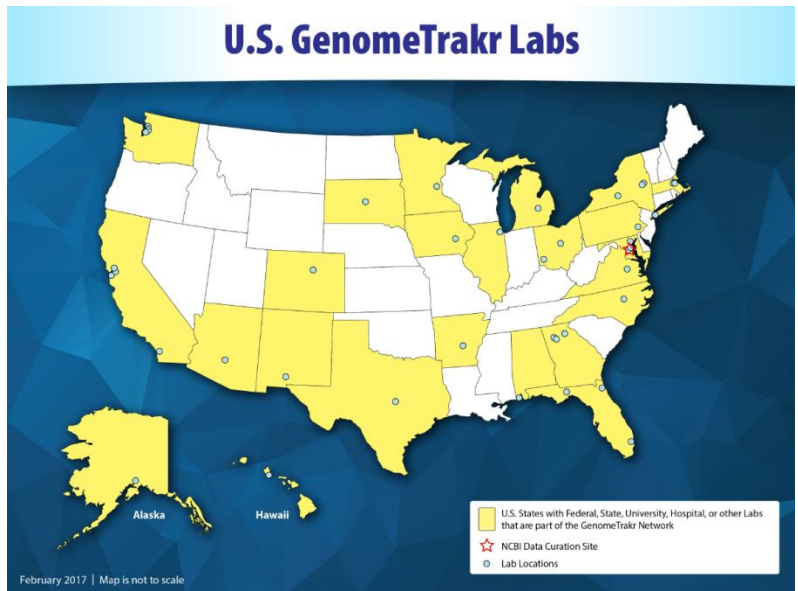
# GenomeTrakr

- Initiated by the FDA to focus on cataloguing, investigating and pinpointing the sources of foodborne pathogens
- 317,000+ isolates sequenced as of Q1 2019
- A network of over 60 GenomeTrakr labs use WGS for pathogen identification
- Data storage and bioinformatics provided by NCBI
- Outputs daily phylogenetic trees for source tracking of isolates
- Can clearly define foodborne illness outbreaks
- Can provide location specificity for outbreak investigations





- 15 federal labs, 25 state health and university labs, 1 US hospital lab, 2 other labs in the US, 20 labs outside the US, collaborations with independent academic researchers (as of 2/25/19)

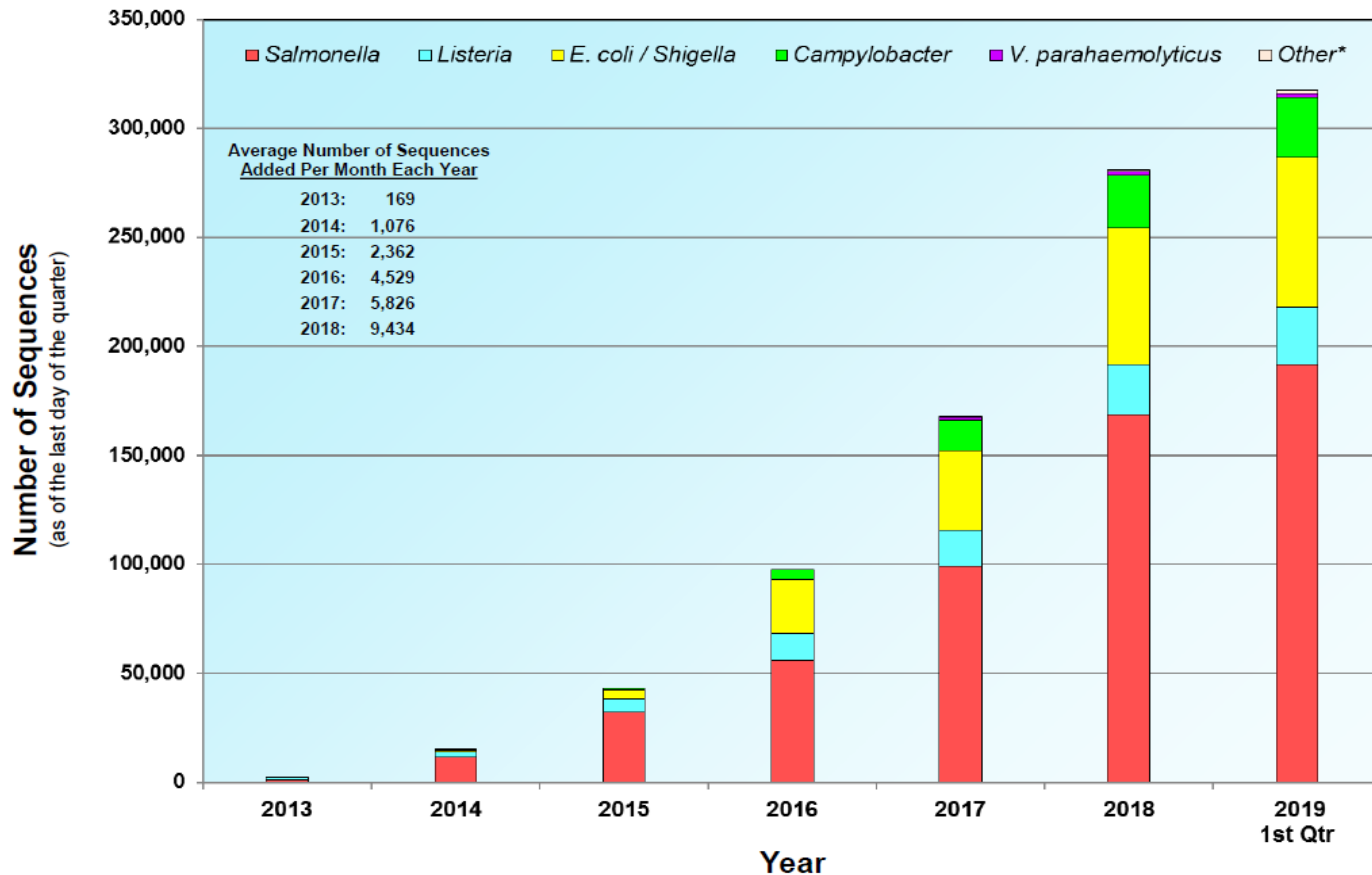


<https://www.fda.gov/food/whole-genome-sequencing-wgs-program/genometrakr-network>





## Total Number of Sequences in the GenomeTrakr Database



First sequences uploaded in February 2013

\* Other pathogens: *Cronobacter*, *V. vulnificus*, *C. botulinum*, and *C. perfringens*

<https://www.fda.gov/food/whole-genome-sequencing-wgs-program/genometrakr-network>





# Government Uses – Assuring Public Health

- Detect outbreaks faster
- Solve more outbreaks while they are still small
- Link epidemiology with exposure data
- Identify new food sources for a given pathogen

# Industry Uses – Traceability

- Pinpoint growth niches
- Solve spoilage issues
- Track problems to suppliers/ingredients





# WGS Use

- CDC began listeriosis surveillance in 2013
  - Sequenced all clinical *L.monocytogenes* isolates
- Has been used routinely in parallel with PFGE
- 2019 – transitioning from PFGE/WGS to WGS exclusively
- Identify pathogens from food, environment or clinical sources
- Establish reliable links between isolates





# WGS Regulatory Applications

- Determine which illnesses are part of an outbreak and which are not
- Determine which ingredient in a food is responsible for an outbreak
- Identify geographic regions where a contaminated ingredient may have originated
- Differentiate sources of contamination within the same outbreak
- Link illnesses to a facility before the implicated food product has been identified
- Link a small number of illnesses that might not have been considered an outbreak
- Identify unlikely routes of contamination

<https://www.fda.gov/food/science-research-food/whole-genome-sequencing-wgs-program>





# Multistate Outbreak of Listeriosis Linked to Roos Foods

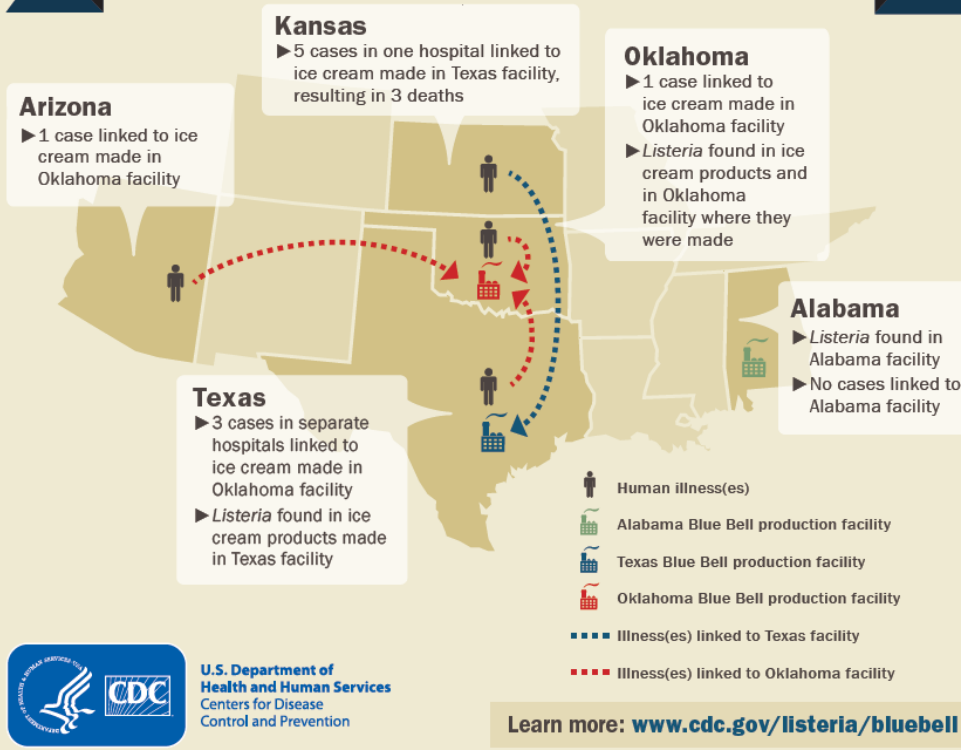
- February 2014
  - One of the first uses of WGS by regulators in outbreak investigations
- *L.monocytogenes* infected 8 people in CA & MD
  - One death in CA
- Mexican soft cheeses
- 12 sponges test positive for *L.monocytogenes*
- Roof leaks, deteriorating areas and equipment
- 8 product types in retail settings test positive for *L.monocytogenes*
- Products recalled
- Shut down for pervasive *L.monocytogenes* in their environment and bad CGMPs
- Products reasonably likely to cause serious adverse health consequences or death



# LISTERIA AND BLUE BELL ICE CREAM

Contaminated production facilities and illnesses linked to Blue Bell Creameries

CDC recommends that people not eat, serve, or sell any recalled Blue Bell brand products. This complicated investigation of a listeriosis outbreak involved serious illnesses from 2010 through 2015 linked to two Blue Bell production facilities.

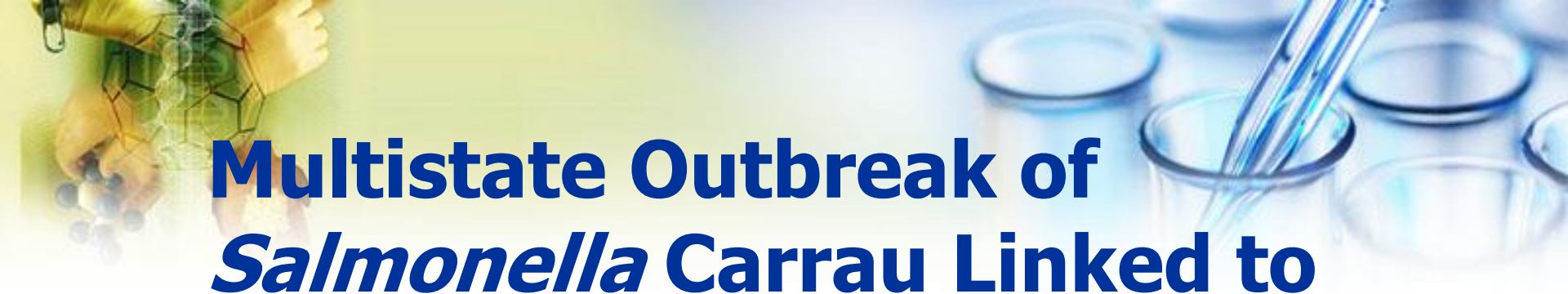


- 10 cases
- 10 hospitalizations
- 4 states
- 3 deaths
- Clinical cases
- Found in 2 products in SC
- TX plant swabbed, 7 different isolates, matched 5 KS cases
- Found in ice cream cups in KS, identified 5 new related cases
- Voluntarily recalled all products

CS257655B

<https://www.cdc.gov/listeria/pdf/bluebell-listeria-outbreak-infographic-508c.pdf>





# Multistate Outbreak of *Salmonella* Carrau Linked to Pre-Cut Melons

- 137 cases in 10 states
- 38 hospitalizations, no deaths
- Clinical isolates closely related
- Epidemiology and traceback evidence indicated pre-cut melons supplied by Caito Foods LLC
- WGS did not identify antibiotic resistance





# Multistate Outbreak of *Salmonella* Concord Linked to Tahini Imported From Israel

- 4 cases in 3 states
- 1 hospitalization, no deaths
- Epidemiology, traceback and lab evidence indicate Karawan brand as the likely source
- This outbreak **not** related to recent multistate outbreak of *Salmonella* Concord infections linked to tahini
- Same serovar but WGS was able to differentiate







# WGS Challenges for Industry

- Need for specially qualified scientists to run this program (if run internally)
- Cost of equipment
- Verifying proficiency
- Cost of sequencing
  - Economy of scale
    - \$1750 – 1 isolate
    - \$1175 – 2-9 isolates
    - \$ 650 – 10-19 isolates
- Interpretation of results
  - Molecular biologist
- Cost is coming down and will at some point be more affordable and turnkey





# Industry Action Items

- Develop a strategy for where and how you will use WGS
  - Tracking and trending in your environment, serology or PFGE are probably sufficient and still provide valuable information
  - Troubleshooting, may want to use WGS
  - FDA inspection, swab where they swab, have your own WGS data if there is a positive
  - Know your environment and what is in it
- Have access to experts in WGS and epidemiology for your crisis management team
  - Identify a credible lab
  - Know where to go for help
  - Know how long WGS takes





# Industry Action Items

- Use meetings like this and other industry consortia to improve understanding of WGS in food safety
  - Continue to develop new ways to use the technology
  - Support research projects to advance the science
- Invest in the future workforce so that they are familiar with genomic tools
  - These people will perform your testing and run your WGS or Next Generation Sequencing (NGS) programs





# Conclusions

- WGS is a powerful tool to aid in outbreak investigations
  - Just a tool, can't stand alone
  - Epidemiological data is still essential
- WGS is a global technology
  - Sequences are being uploaded around the world daily
  - A lot of our food is imported, important to use WGS to your advantage
- WGS is no longer on the horizon, it's here
  - Continuing education is key
  - Stay abreast of developments and make any necessary adjustments to your food safety plans





# Thank You!

Special thanks to Dr. Laurie Post

