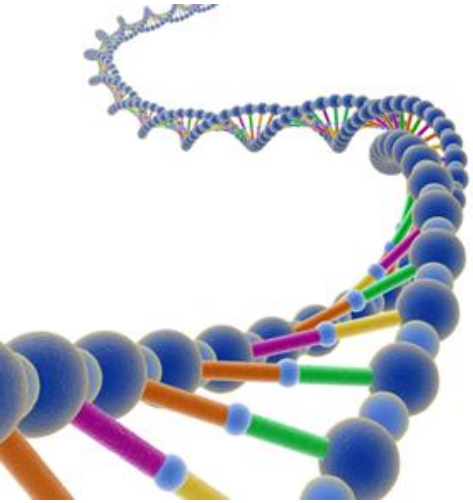
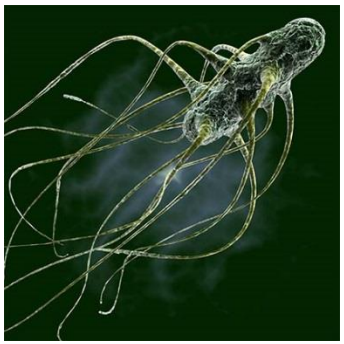
How The FDA Uses WGS for Regulatory Purposes



Maria Hoffmann, Ph.D.

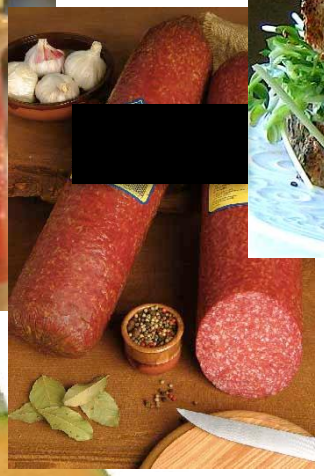
Division of Microbiology
Center for Food Safety & Applied Nutrition
U.S. Food & Drug Administration
College Park, Maryland



May 23, 2017 – Food Sure Summit

Safe, Wholesome, Sanitary Foods

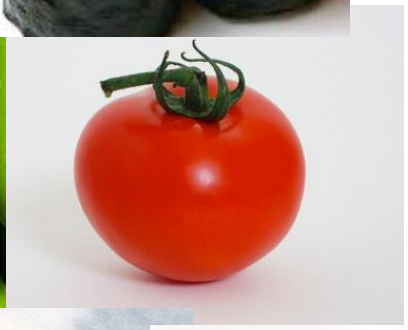
The Salmonella 'radiation'







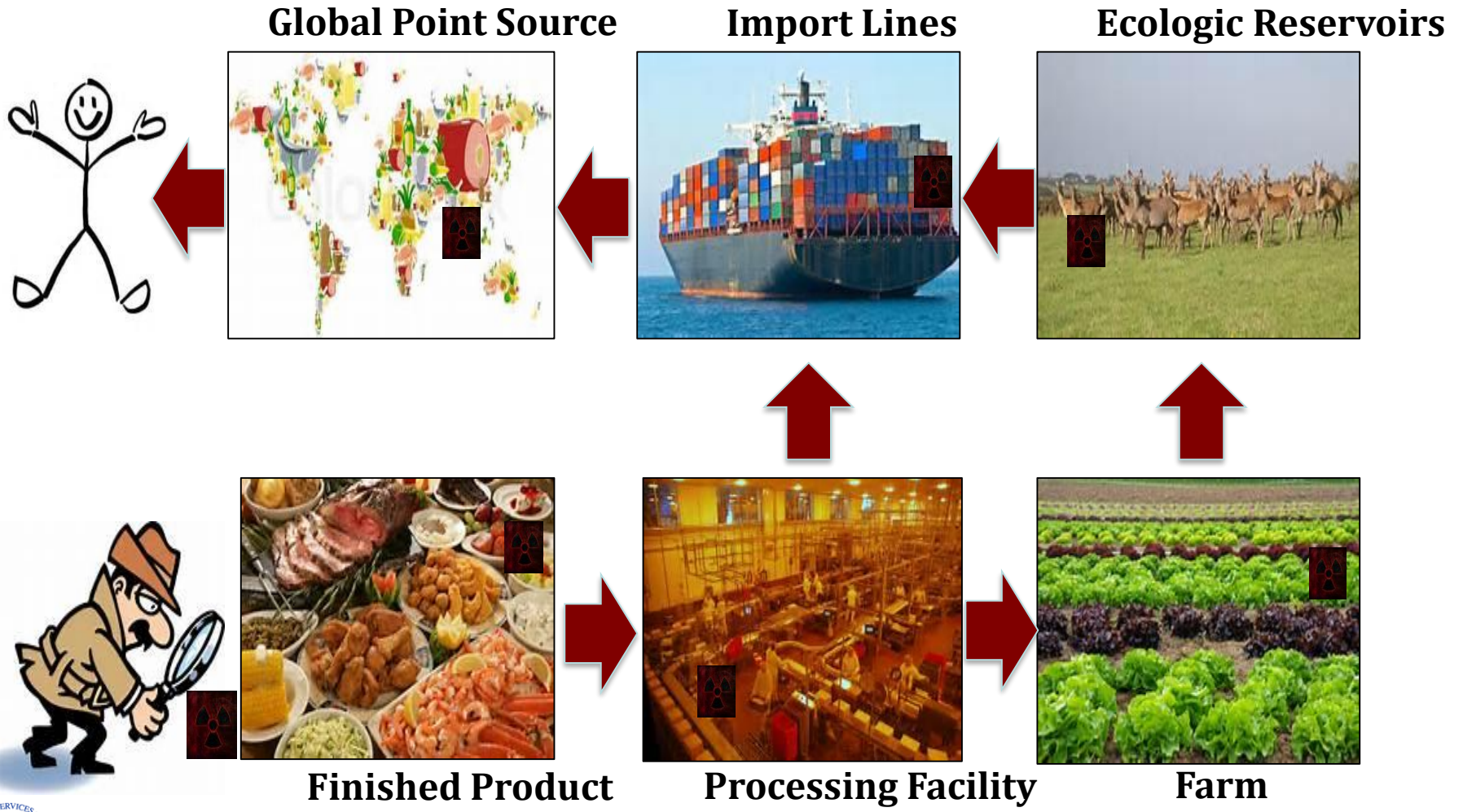






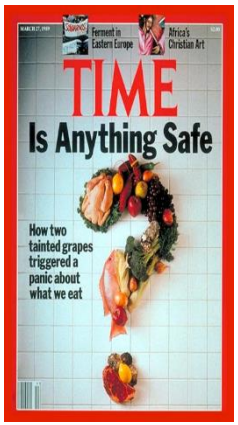



Tracking contamination down…and FAST!

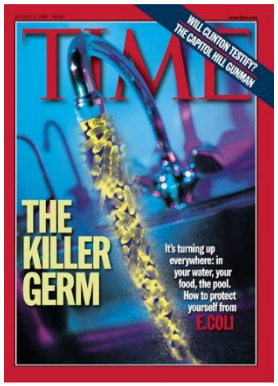


SAVES LIVES

Battling Foodborne Illness



- Each year nearly 10 million episodes of foodborne illness in the United States
- ~60,000 hospitalizations
- More than 1300 deaths



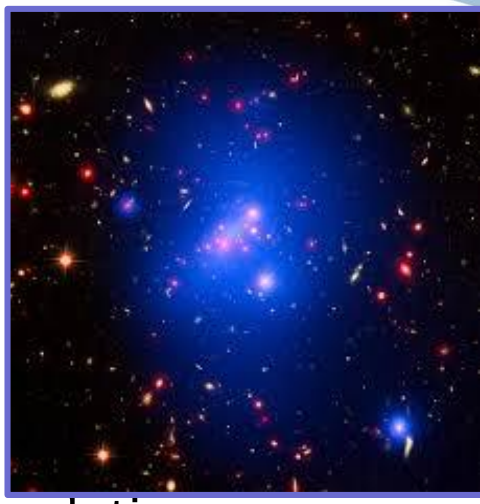
- *Salmonella spp. cause* 11% of foodborne illnesses each year

(Scallan et al. 2011 Emerging Infectious Diseases • www.cdc.gov/eid).

Some perspective on the food supply

- **Tracking and Tracing of food pathogens**
  - Almost 200,000 registered food facilities (2/14)
    - 81,574 Domestic and 115,753 Foreign
  - More than 300 ports of entry
  - More than 130,000 importers and more than 11 million import lines/yr
  - In the US there are more than 2 million farms

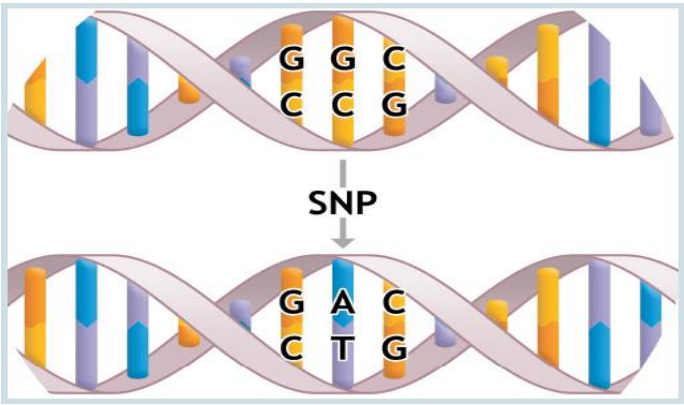
PFGE transition to WGS



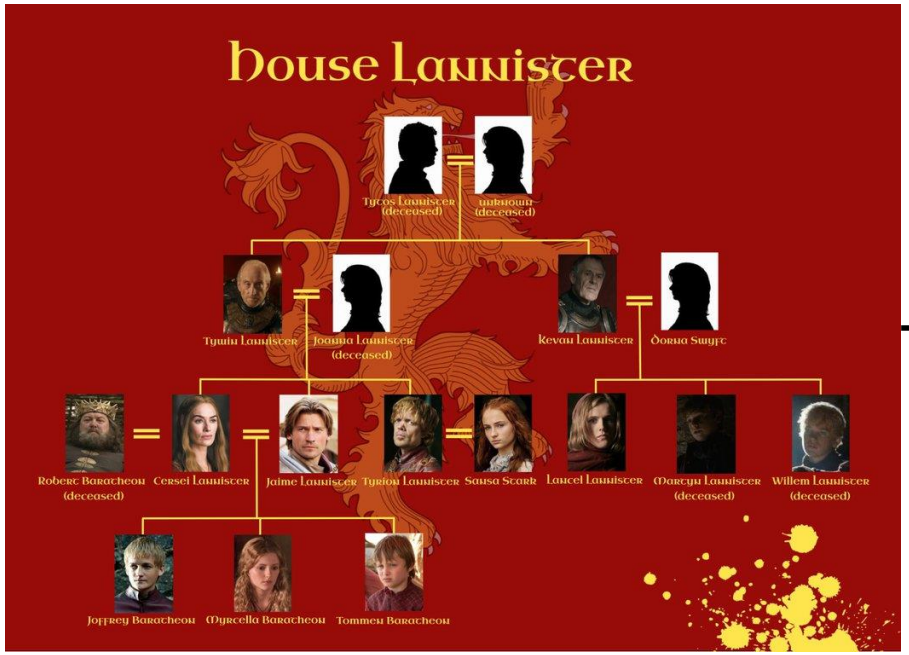
- WGS is high resolution
  - 3-5 million data points are collected for each isolate

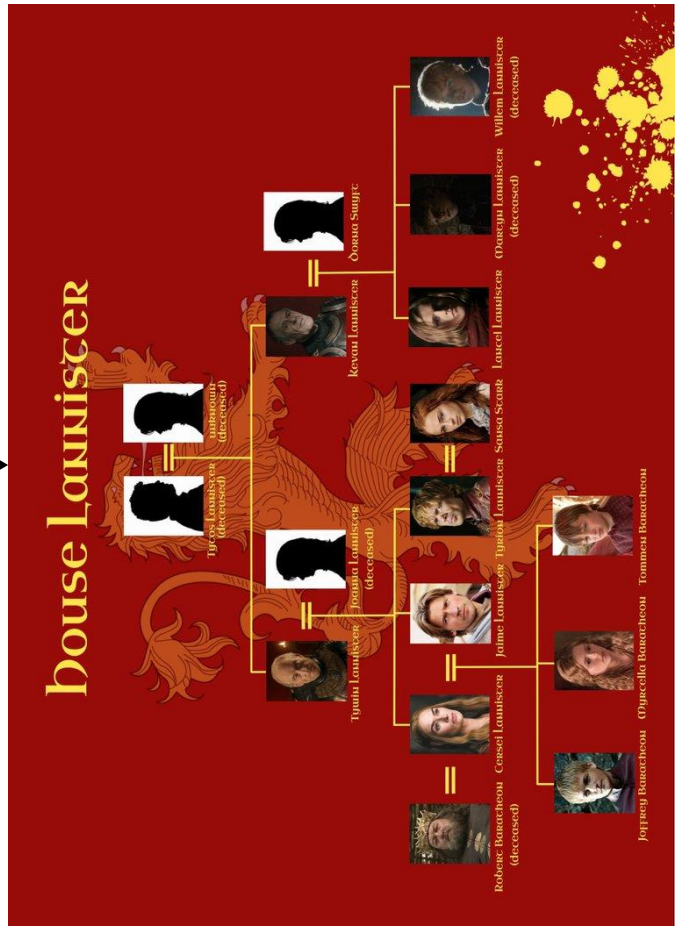
- WGS analyses are statistically robust
  - Unlike PFGE patterns, WGS data can be analyzed in its evolutionary context. Accurate and stable genetic changes within pathogen genomes enable us to pin point specific common sources of outbreak strains (farms, processing plants, food types, and geographic regions).

  - ***Source Tracking is Key Application: moving from PFGE to WGS has been no less impactful than the move from backyard telescopes to the Hubble in terms of resolution in differentiating foodborne outbreaks.***



Pedigree vs Phylogeny





DNA Forensics of Microbes

Genome sequence is agnostic. One biological assay could works on all pathogen species

Immediately useful. All we need is the genome and a little metadata.

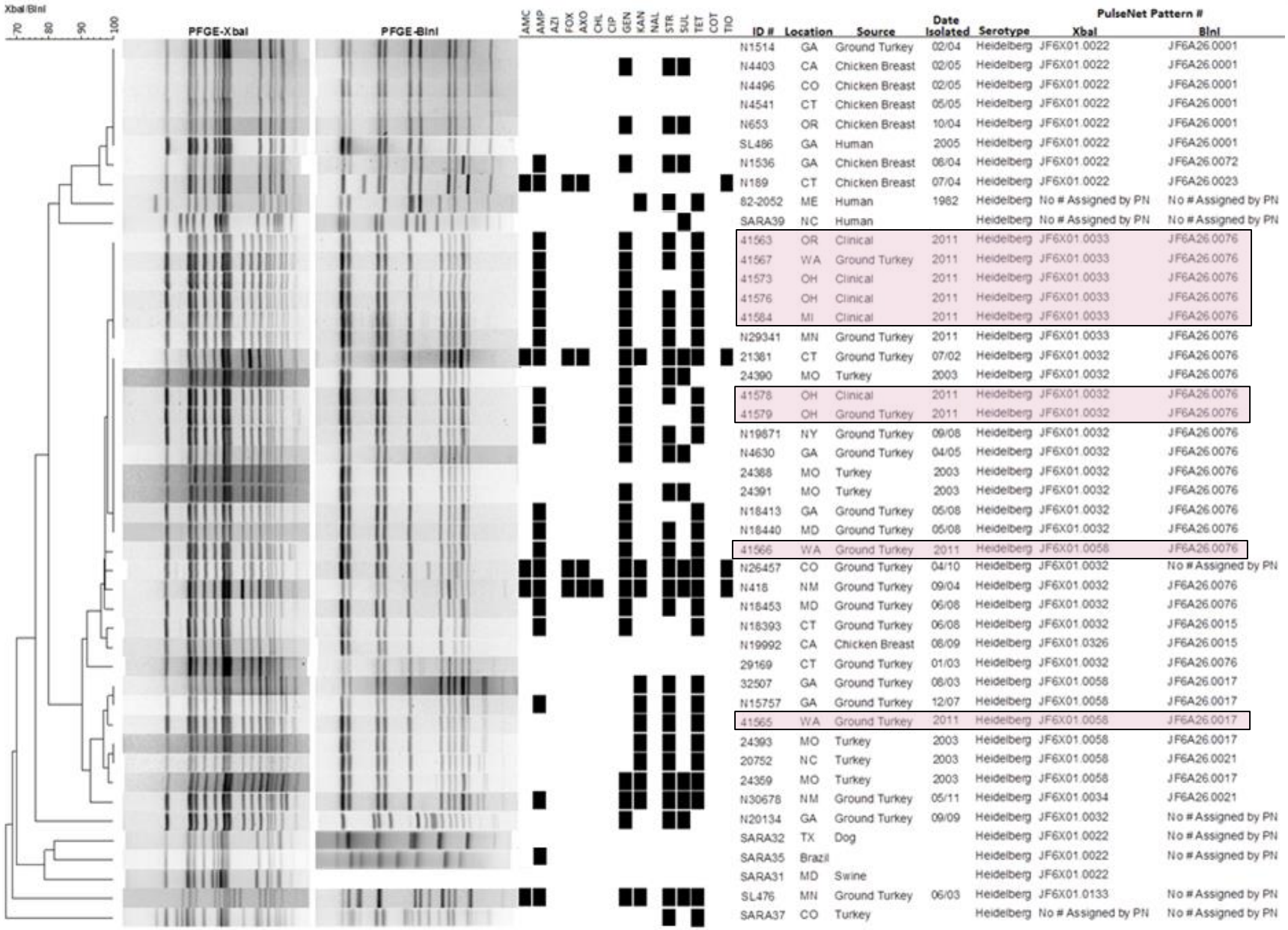
WGS is now routine in FDA's outbreak response and compliance/surveillance activities. Internally (across our agency), and in collaboration with FSIS and CDC, WGS has now been deployed and benefitted the traceability of numerous foodborne contamination events.

WGS can be used to inform traceback investigations and delimit the scope of food contamination events unlike ever before – not just a regulatory tool - numerous offshoot applications exist (i.e., supply chain management, quality assurance, process evaluation, etc.)

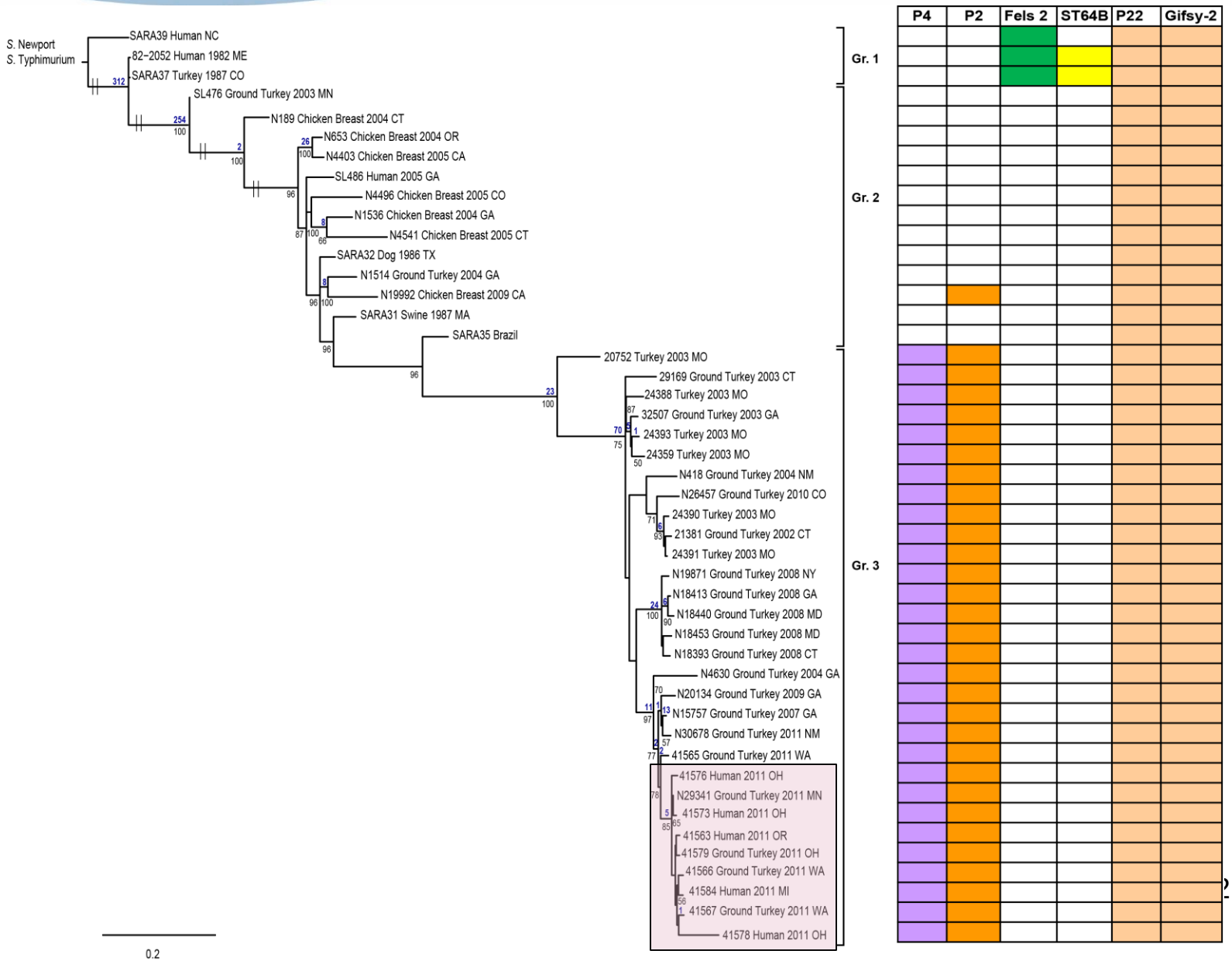
A Pathogen Genome Is The Fingerprint

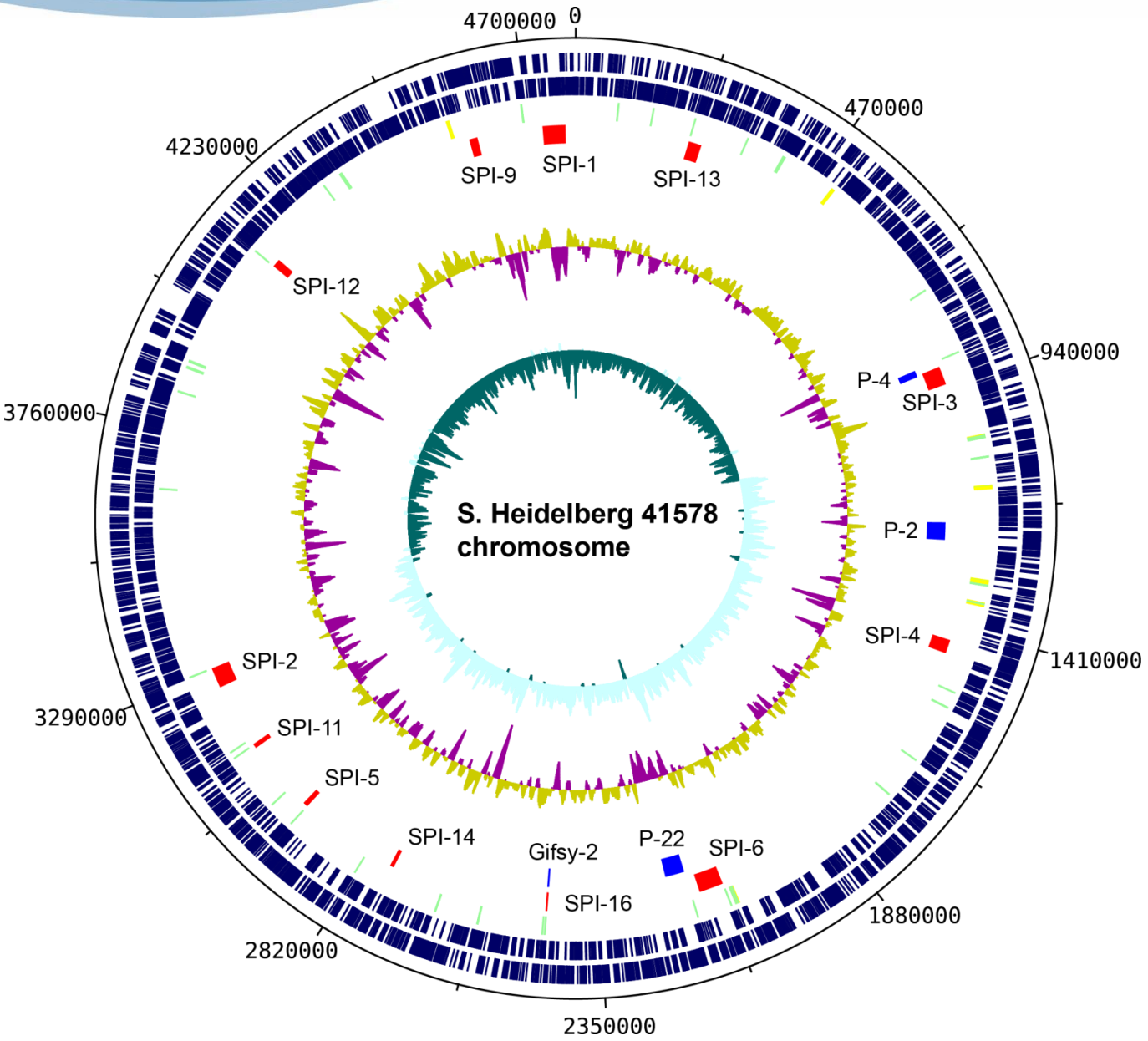
Salmonella enterica serovar Heidelberg

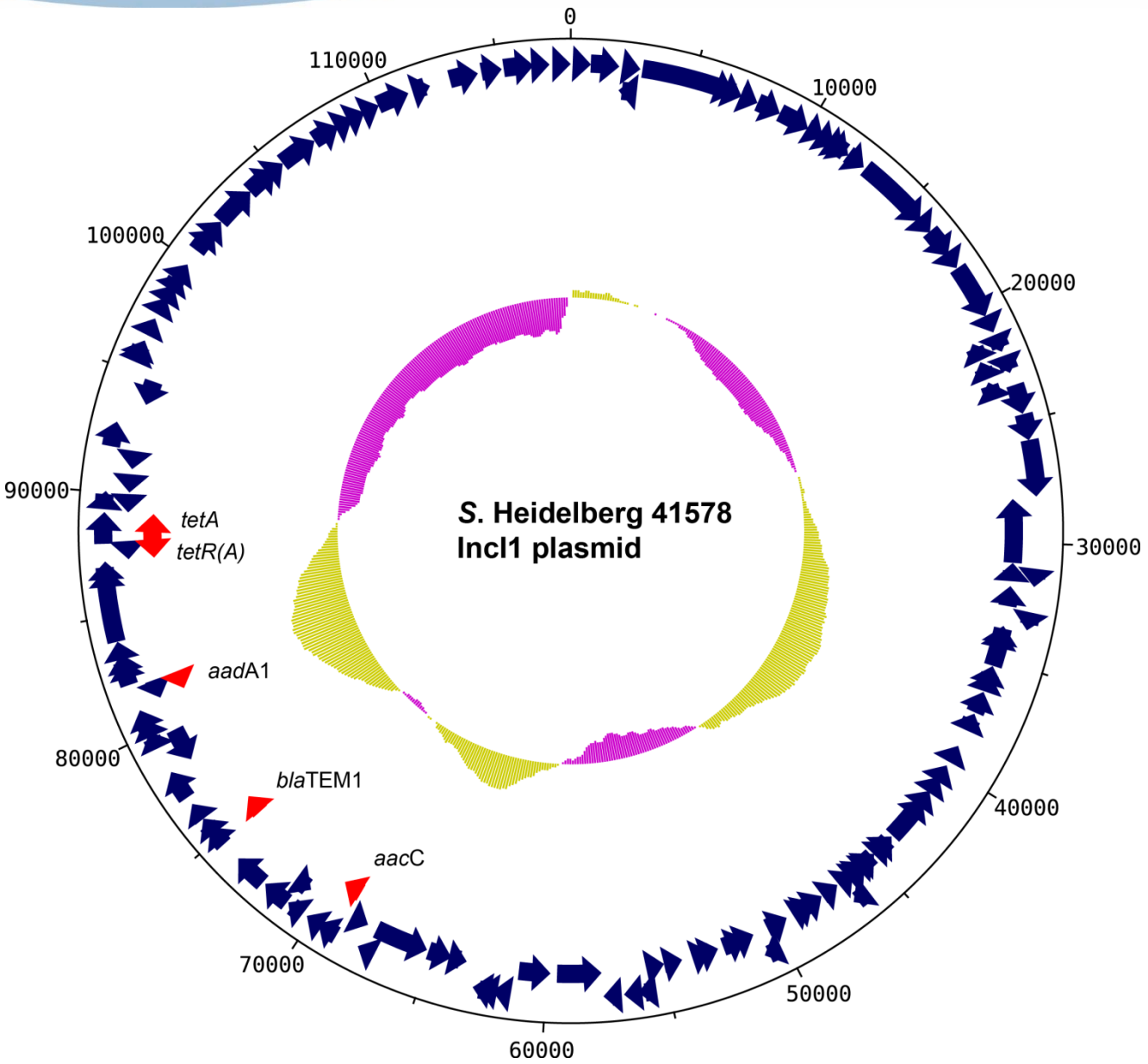
- CDC investigated a multistate (34 states) outbreak
- 136 confirmed cases between February 27th and September 13th, 2011
- Among the 94 case patient, 37 (39%) had been hospitalized and one patient died
- GT was implicated as source of this outbreak
- Largest product recall in history of 36 million tons of GT meat

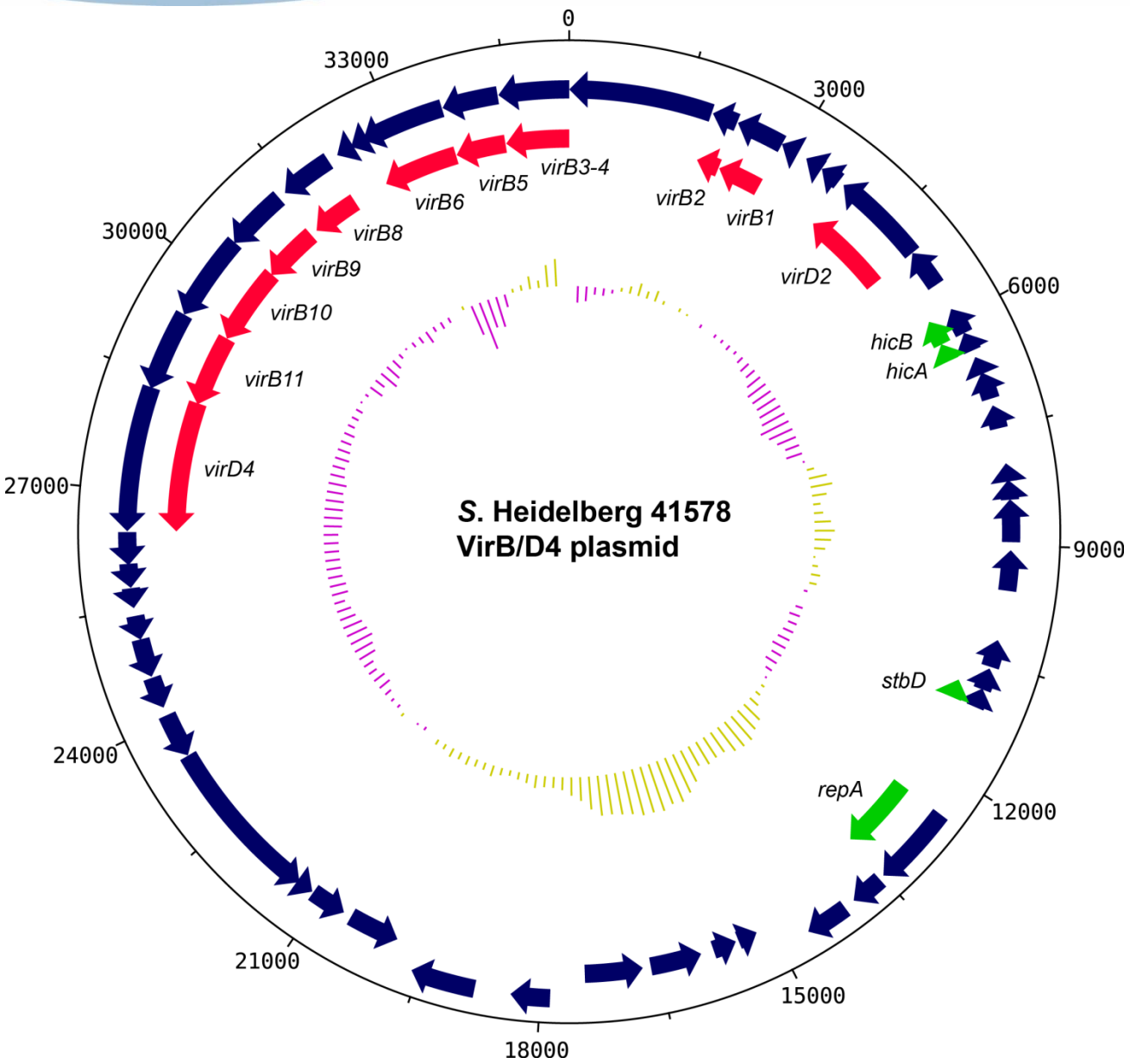


ID #	Location	Source	Date Isolated	Serotype	PulseNet Pattern # XbaI	PulseNet Pattern # BlnI
N1514	GA	Ground Turkey	02/04	Heidelberg	JF6X01.0022	JF6A26.0001
N4403	CA	Chicken Breast	02/05	Heidelberg	JF6X01.0022	JF6A26.0001
N4496	CO	Chicken Breast	02/05	Heidelberg	JF6X01.0022	JF6A26.0001
N4541	CT	Chicken Breast	05/05	Heidelberg	JF6X01.0022	JF6A26.0001
N653	OR	Chicken Breast	10/04	Heidelberg	JF6X01.0022	JF6A26.0001
SL486	GA	Human	2005	Heidelberg	JF6X01.0022	JF6A26.0001
N1536	GA	Chicken Breast	06/04	Heidelberg	JF6X01.0022	JF6A26.0072
N189	CT	Chicken Breast	07/04	Heidelberg	JF6X01.0022	JF6A26.0023
82-2052	ME	Human	1982	Heidelberg	No # Assigned by PN	No # Assigned by PN
SARA39	NC	Human		Heidelberg	No # Assigned by PN	No # Assigned by PN
41563	OR	Clinical	2011	Heidelberg	JF6X01.0033	JF6A26.0076
41567	WA	Ground Turkey	2011	Heidelberg	JF6X01.0033	JF6A26.0076
41573	OH	Clinical	2011	Heidelberg	JF6X01.0033	JF6A26.0076
41576	OH	Clinical	2011	Heidelberg	JF6X01.0033	JF6A26.0076
41584	MI	Clinical	2011	Heidelberg	JF6X01.0033	JF6A26.0076
N29341	MN	Ground Turkey	2011	Heidelberg	JF6X01.0033	JF6A26.0076
21381	CT	Ground Turkey	07/02	Heidelberg	JF6X01.0032	JF6A26.0076
24390	MO	Turkey	2003	Heidelberg	JF6X01.0032	JF6A26.0076
41578	OH	Clinical	2011	Heidelberg	JF6X01.0032	JF6A26.0076
41579	OH	Ground Turkey	2011	Heidelberg	JF6X01.0032	JF6A26.0076
N19871	NY	Ground Turkey	09/08	Heidelberg	JF6X01.0032	JF6A26.0076
N4630	GA	Ground Turkey	04/05	Heidelberg	JF6X01.0032	JF6A26.0076
24388	MO	Turkey	2003	Heidelberg	JF6X01.0032	JF6A26.0076
24391	MO	Turkey	2003	Heidelberg	JF6X01.0032	JF6A26.0076
N18413	GA	Ground Turkey	05/08	Heidelberg	JF6X01.0032	JF6A26.0076
N18440	MD	Ground Turkey	05/08	Heidelberg	JF6X01.0032	JF6A26.0076
41566	WA	Ground Turkey	2011	Heidelberg	JF6X01.0058	JF6A26.0076
N26457	CO	Ground Turkey	04/10	Heidelberg	JF6X01.0032	No # Assigned by PN
N418	NM	Ground Turkey	09/04	Heidelberg	JF6X01.0032	JF6A26.0076
N18453	MD	Ground Turkey	06/08	Heidelberg	JF6X01.0032	JF6A26.0076
N18393	CT	Ground Turkey	06/08	Heidelberg	JF6X01.0032	JF6A26.0015
N19992	CA	Chicken Breast	06/09	Heidelberg	JF6X01.0326	JF6A26.0015
29169	CT	Ground Turkey	01/03	Heidelberg	JF6X01.0032	JF6A26.0076
32507	GA	Ground Turkey	06/03	Heidelberg	JF6X01.0058	JF6A26.0017
N15757	GA	Ground Turkey	12/07	Heidelberg	JF6X01.0058	JF6A26.0017
41565	WA	Ground Turkey	2011	Heidelberg	JF6X01.0058	JF6A26.0017
24393	MO	Turkey	2003	Heidelberg	JF6X01.0058	JF6A26.0017
20752	NC	Turkey	2003	Heidelberg	JF6X01.0058	JF6A26.0021
24359	MO	Turkey	2003	Heidelberg	JF6X01.0058	JF6A26.0017
N30678	NM	Ground Turkey	05/11	Heidelberg	JF6X01.0034	JF6A26.0021
N20134	GA	Ground Turkey	09/09	Heidelberg	JF6X01.0032	No # Assigned by PN
SARA32	TX	Dog		Heidelberg	JF6X01.0022	No # Assigned by PN
SARA35	Brazil			Heidelberg	JF6X01.0022	No # Assigned by PN
SARA31	MD	Swine		Heidelberg	JF6X01.0022	
SL476	MN	Ground Turkey	06/03	Heidelberg	JF6X01.0133	No # Assigned by PN
SARA37	CO	Turkey		Heidelberg	No # Assigned by PN	No # Assigned by PN


S. Newport
S. Typhimurium
SARA39 Human NC
82-2052 Human 1982 ME
SARA37 Turkey 1987 CO
SL476 Ground Turkey 2003 MN
N189 Chicken Breast 2004 CT
N653 Chicken Breast 2004 OR
N4403 Chicken Breast 2005 CA
SL486 Human 2005 GA
N4496 Chicken Breast 2005 CO
N1536 Chicken Breast 2004 GA
N4541 Chicken Breast 2005 CT
SARA32 Dog 1986 TX
N1514 Ground Turkey 2004 GA
N19992 Chicken Breast 2009 CA
SARA31 Swine 1987 MA
SARA35 Brazil
20752 Turkey 2003 MO
29169 Ground Turkey 2003 CT
24388 Turkey 2003 MO
32507 Ground Turkey 2003 GA
24393 Turkey 2003 MO
24359 Turkey 2003 MO
N418 Ground Turkey 2004 NM
N26457 Ground Turkey 2010 CO
24390 Turkey 2003 MO
21381 Ground Turkey 2002 CT
24391 Turkey 2003 MO
N19871 Ground Turkey 2008 NY
N18413 Ground Turkey 2008 GA
N18440 Ground Turkey 2008 MD
N18453 Ground Turkey 2008 MD
N18393 Ground Turkey 2008 CT
N4630 Ground Turkey 2004 GA
N20134 Ground Turkey 2009 GA
N15757 Ground Turkey 2007 GA
N30678 Ground Turkey 2011 NM
41565 Ground Turkey 2011 WA
41576 Human 2011 OH
N29341 Ground Turkey 2011 MN
41573 Human 2011 OH
41563 Human 2011 OR
41579 Ground Turkey 2011 OH
41566 Ground Turkey 2011 WA
41584 Human 2011 MI
41567 Ground Turkey 2011 WA
41578 Human 2011 OH
Gr. 1
Gr. 2
Gr. 3
0.2
P4
P2
Fels 2
ST64B
P22
Gifsy-2


4700000
0
470000
940000
1410000
1880000
2350000
2820000
3290000
3760000
4230000
SPI-9
SPI-1
SPI-13
SPI-12
P-4
SPI-3
P-2
SPI-4
SPI-2
SPI-11
SPI-5
SPI-14
Gifsy-2
SPI-16
P-22
SPI-6
S. Heidelberg 41578
chromosome


0
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
110000
S. Heidelberg 41578
IncI1 plasmid
tetA
tetR(A)
aadA1
blaTEM1
aacC

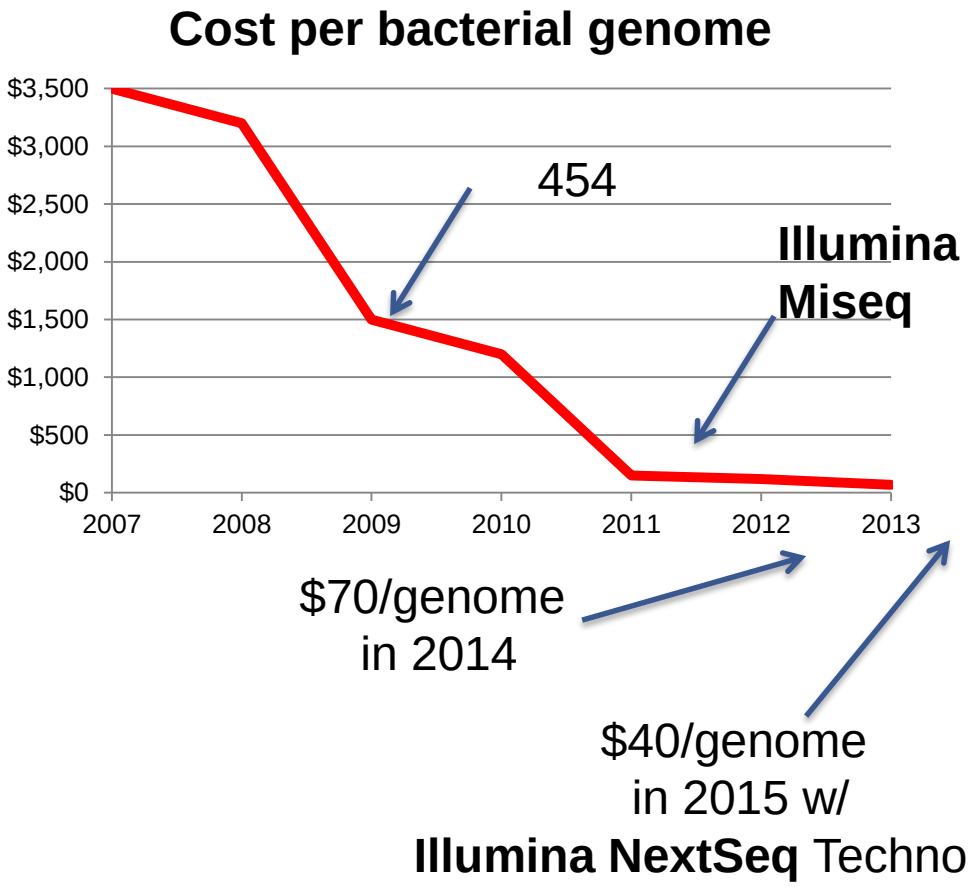

0
3000
6000
9000
12000
15000
18000
21000
24000
27000
30000
33000
virB3-4
virB5
virB6
virB8
virB9
virB10
virB11
virD4
virB2
virB1
virD2
hicB
hicA
stbD
repA
S. Heidelberg 41578
VirB/D4 plasmid

"Whole Genome Sequencing Is The Biggest Thing To Happen To Food Microbiology Since Pasteur Showed Us How To Culture Pathogens…"

Dr. Jorgen Schlundt
Exec Director and Founder
The Global Microbial Identifier

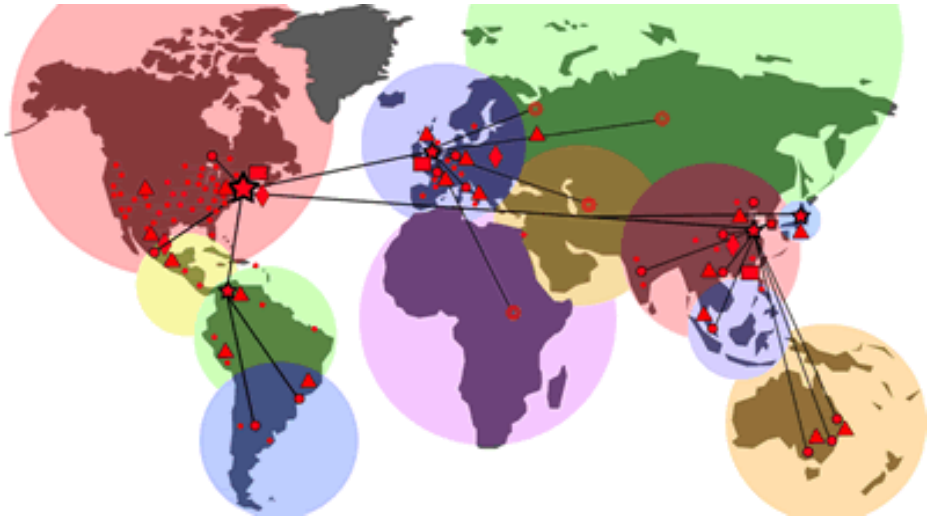
Is WGS a viable solution?

- Cost
- Increasing ease of operation
- Database longevity
- Comparable times to conventional pipelines
- Sample prep
  - Identical for all pathogens
- Cost savings
  - Resistance, subtyping, virulence factors, more…
- New applications
  - tracking, regulatory/compliance actions, historical trends, more…

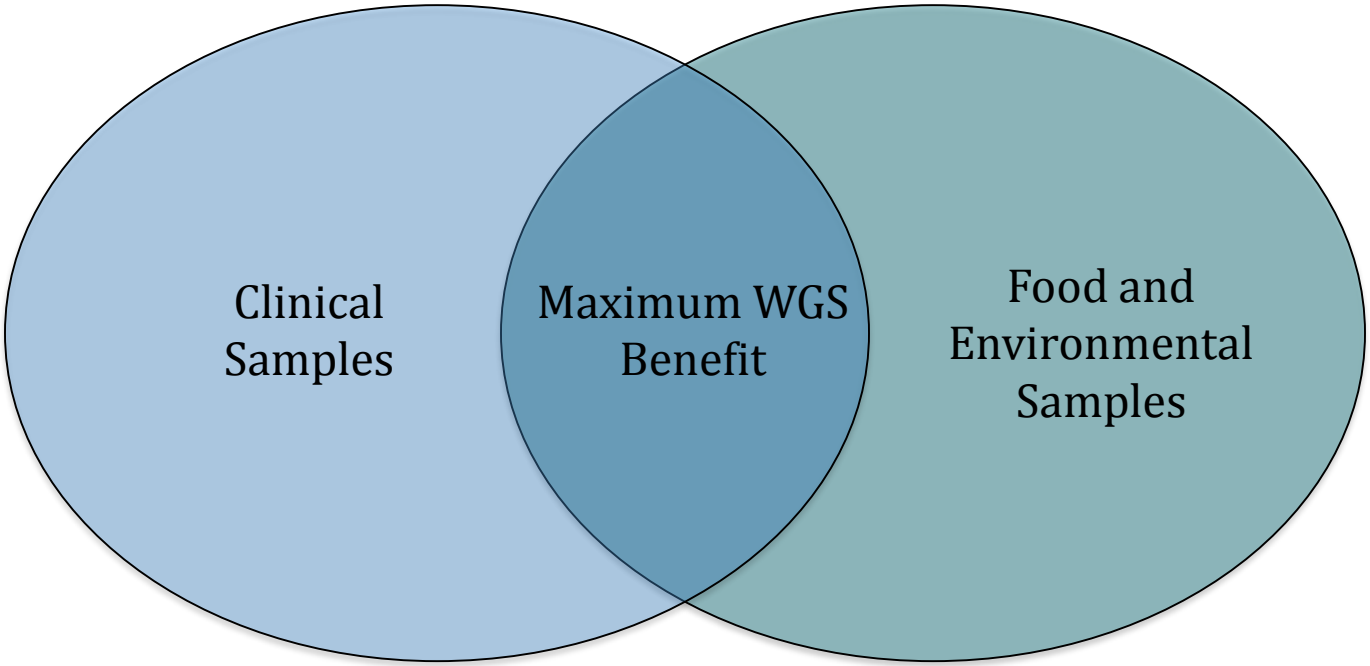


Why Develop a WGS Based Network?

- Tracking and Tracing of food pathogens
  - Insufficient resolution of current tools
    -matching clinical to environmental
  - Faster identification of the food involved in the outbreak
  - Limited number of investigators vs. facilities and import lines
  - Global travel
  - Global food supply



Importance of a Balanced Approach



20 years of PulseNet USA
1996 - 2016

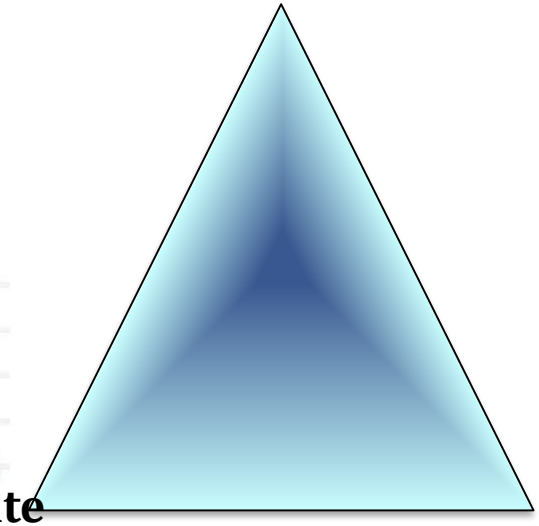
GENOME TRAKR

Key elements of a national/international WGS network

NCBI/EMBL/DDBJ

Sequence storage,
data provider & preliminary analysis

Labs to generate WGS data



Network management

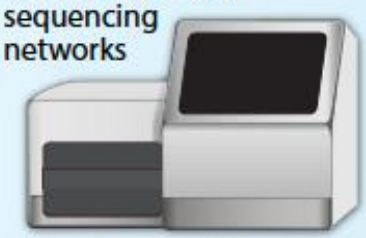
Basic Data Flow for Global WGS Public Access Databases

DATA ACQUISITION

Sequence and upload genomic and geographic data

GENOME TRAKR

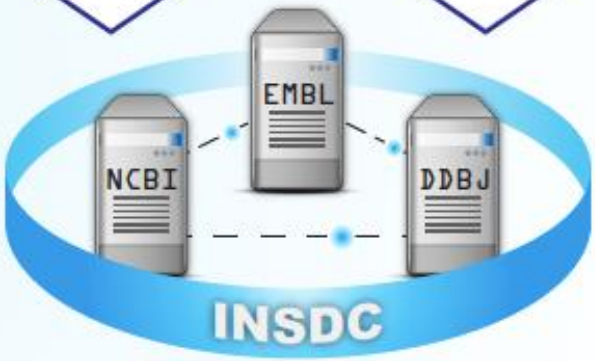
Other distributed sequencing networks



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*



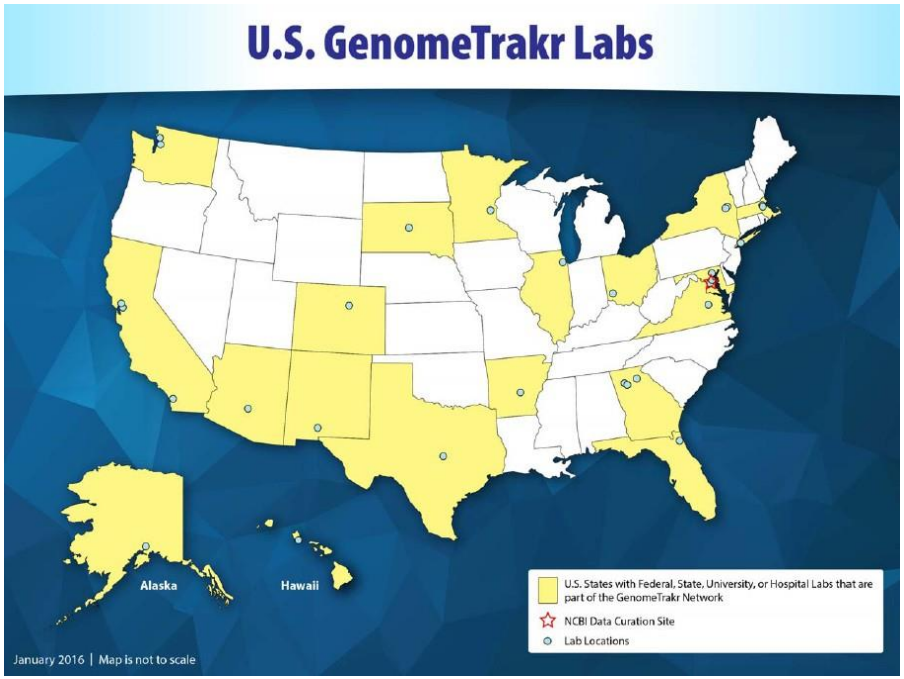
PUBLIC HEALTH APPLICATION AND INTERPRETATION OF DATA

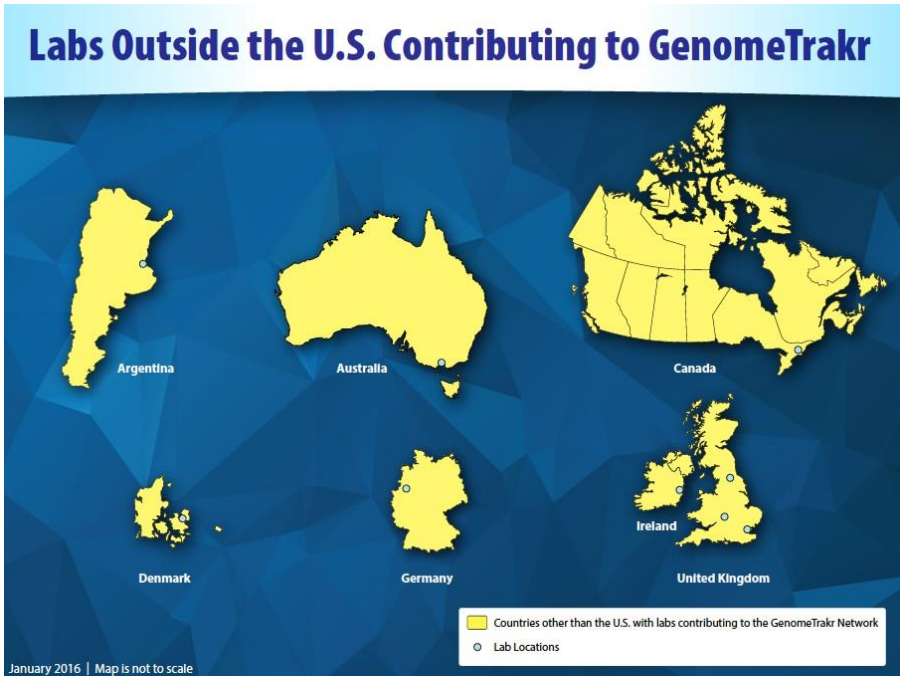
- *Find clinical links*
- *Identify clusters*
- *Conduct traceback*
- *Develop rapid methods*
- *Develop culture independent tests*
- *Develop new analytical software*

State, Local, Federal, and Foreign Public Health Agencies

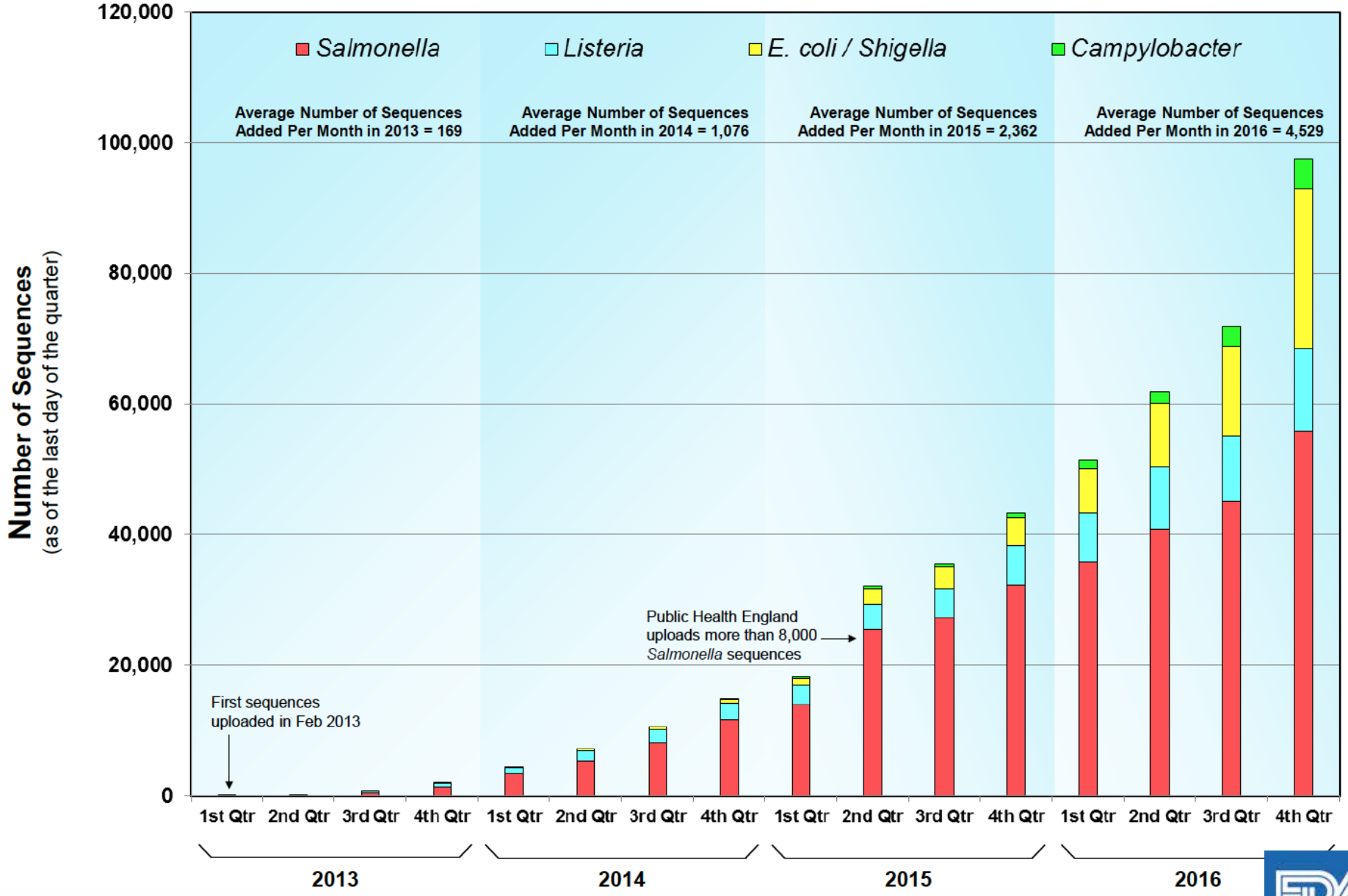
Academia/Industry

58 Participating Laboratories



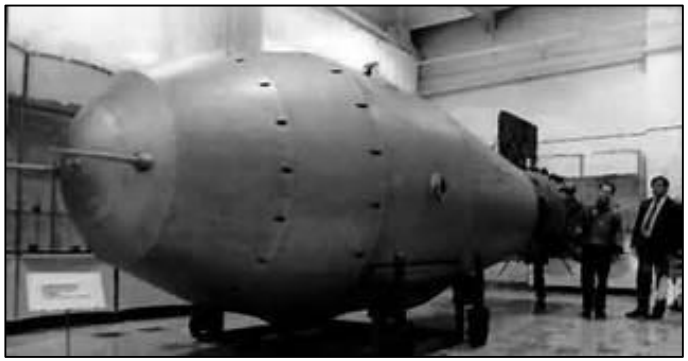


Total Number of Sequences in the GenomeTrakr Database



"Open Data" paradigm

- Raw genome sequences made available to the public 1-2 days after collection
  - Isolates from routine FDA + State Lab lab inspections
  - Isolates from historical collections, past outbreaks
  - Environmental monitoring/surveillance
  - Routine food sampling for imports
- Data made public *before* FDA analyses are performed



"If they could not keep the data associated with the development of the atomic bomb a secret, how possibly do you think you can keep whole genome sequencing data secret? It will come out eventually."

Dr. Jorgen Schlundt
Exec Director and Founder
The Global Microbial Identifier

What happens with a WGS link between a clinical and environmental sample?

- Likely result in the following steps:

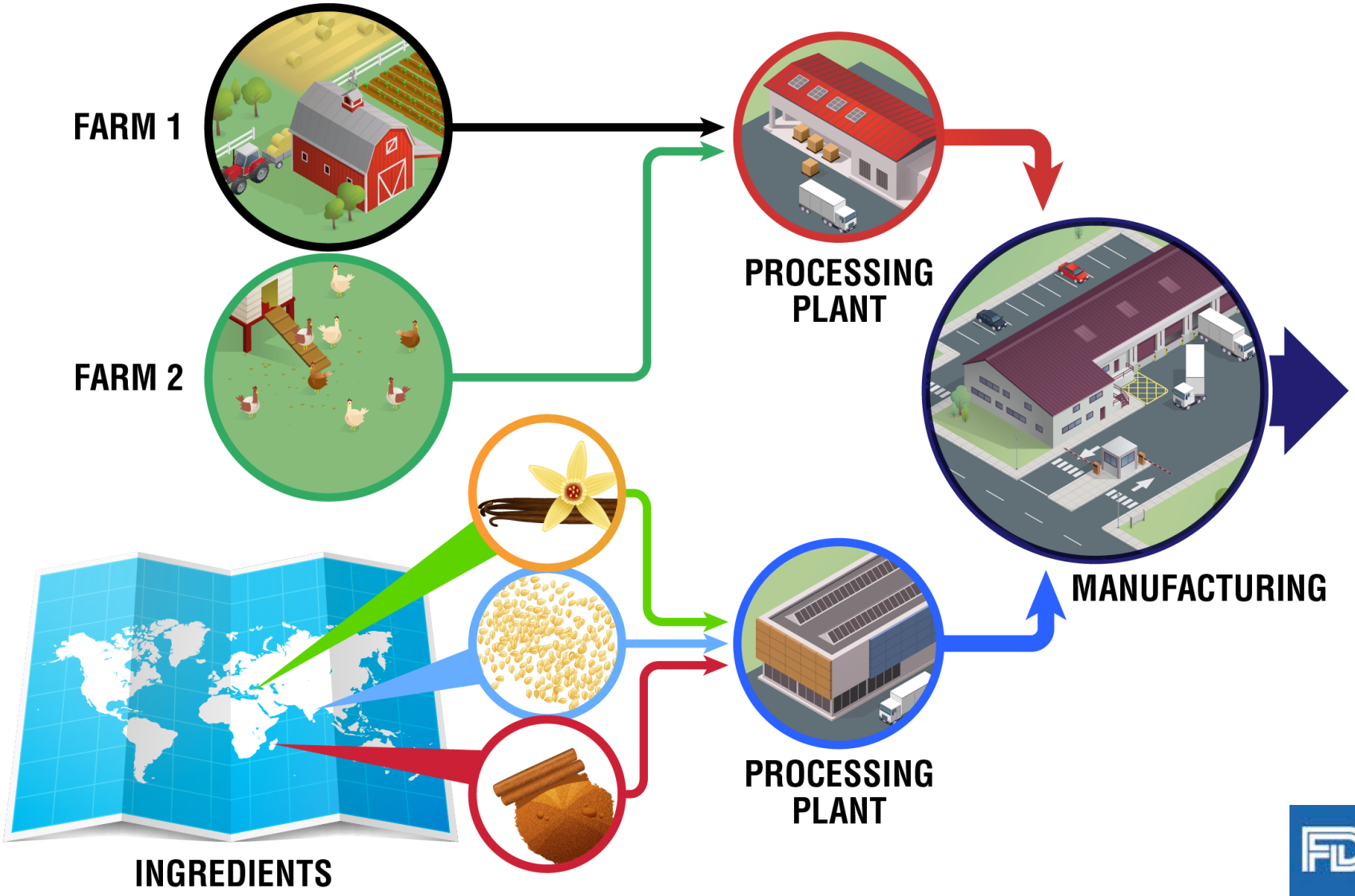
  (1) facility/farm follow-up and sampling

  (2) Additional pathogen positive samples are sequenced and submitted to the database

  (3) In case of outbreak, supports epidemiology and traceback/trace forward of raw materials and finished product

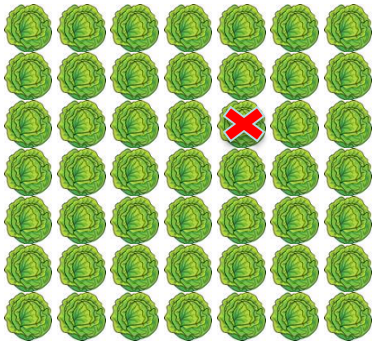
  (4) WGS is powerful tool that supports investigation

Environmental sampling combined with WGS can help point to root cause of the contamination

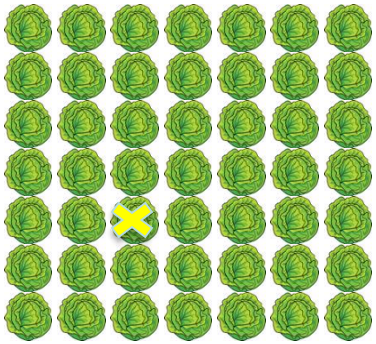


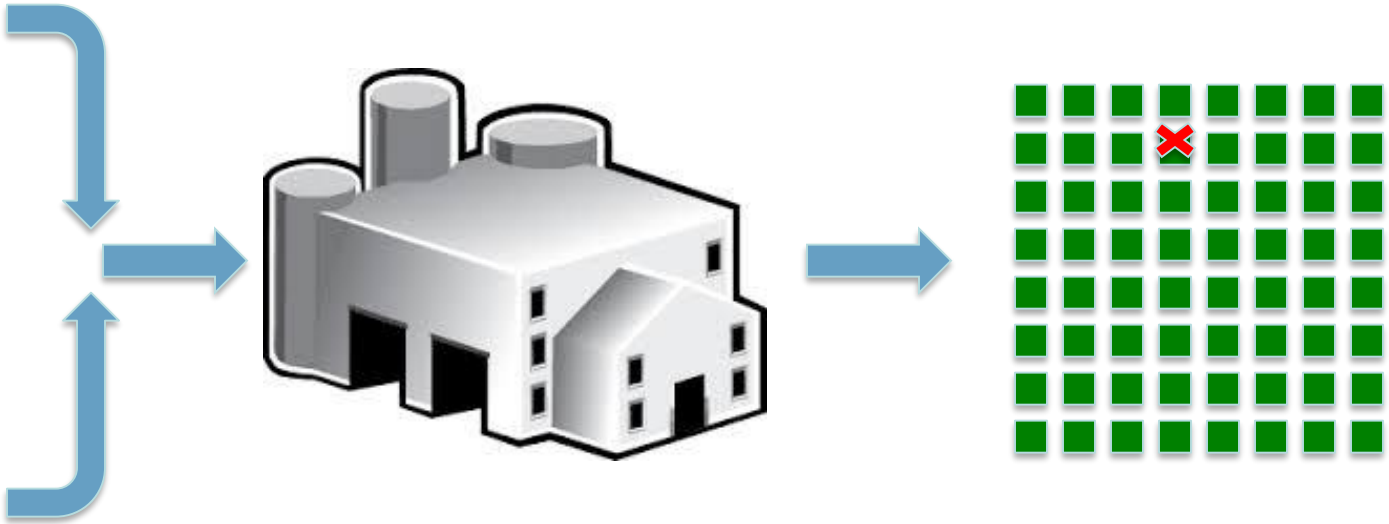
Scenario 1 (pass through)

Field 1



Field 2

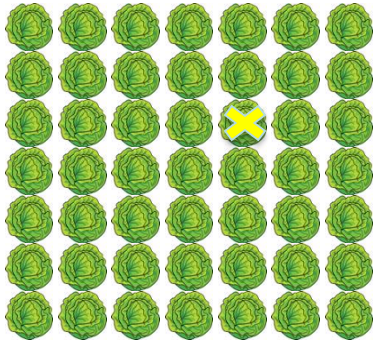




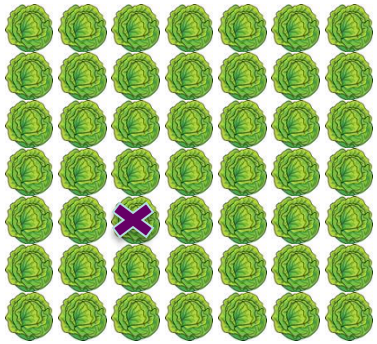
Processing facility

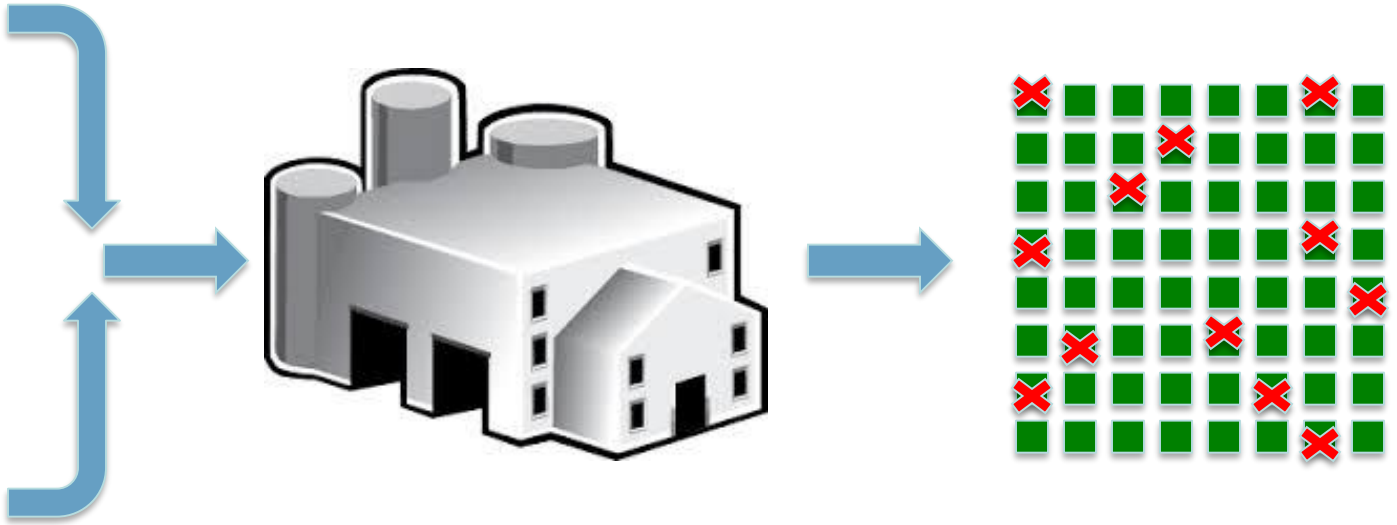
Scenario 2 (harborage and persistence)

Field 1



Field 2





Processing facility

Regular testing throughout network

- Identifies specific suppliers that are introducing contaminants
- Identifies whether contaminant is resident to a facility or transient
- Knowledge of where contaminant is coming from allows industry to fix the problem based on scientific evidence.

Next-Generation sequencing can be used to address different facets of outbreak response:

- **Have we seen this isolate before?**
  (Compare it to reference isolates)
- **Do these isolates form a cluster? (i.e. is it outbreak or background**
  (Compare them to reference and other outbreak isolates)
- **Is there a similarity between food/environmental and clinical isolates?**
  (Compare them to reference, clinical, and food/environmental isolates)



Same PFGE pattern
10-15 SNPs

2014 outbreak
5 SNPs

Real-time Integration of WGS into FDA regulatory workflow

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

March 11, 2014

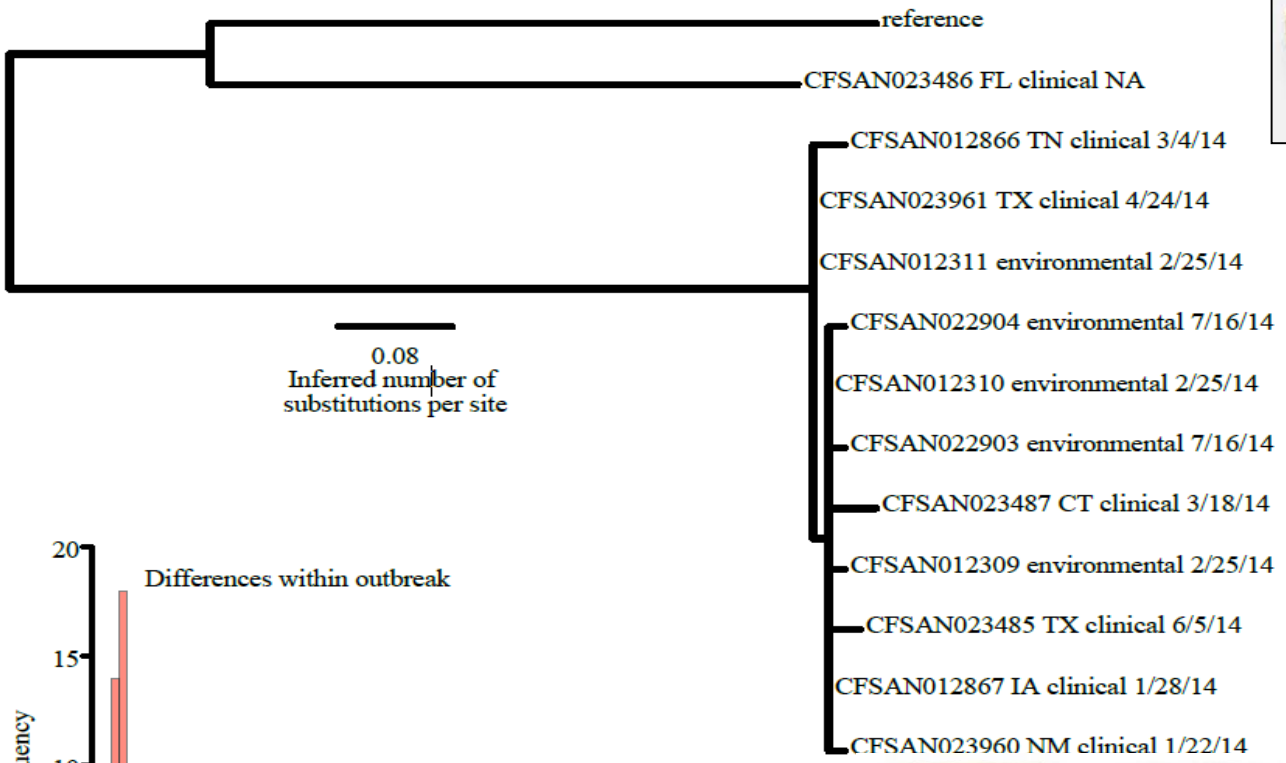
[redacted]t
Roos Foods Inc.
251 Roos Lane
Kenton, DE 19955

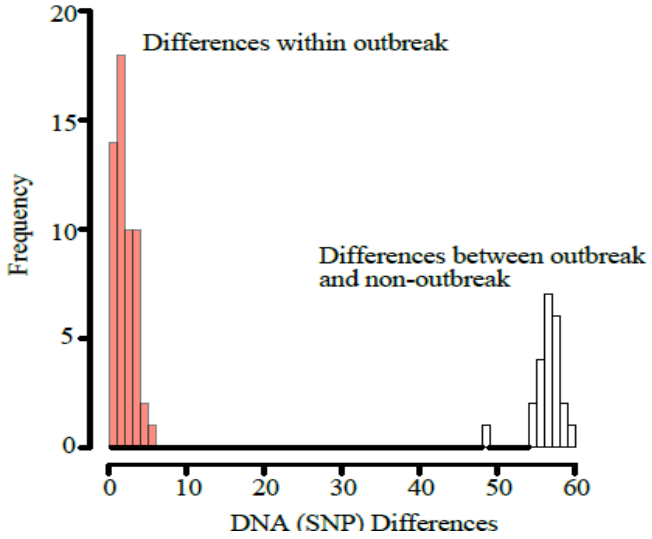
ORDER: Suspension of Food Facility Registration
Notice of Opportunity for Hearing

[redacted]

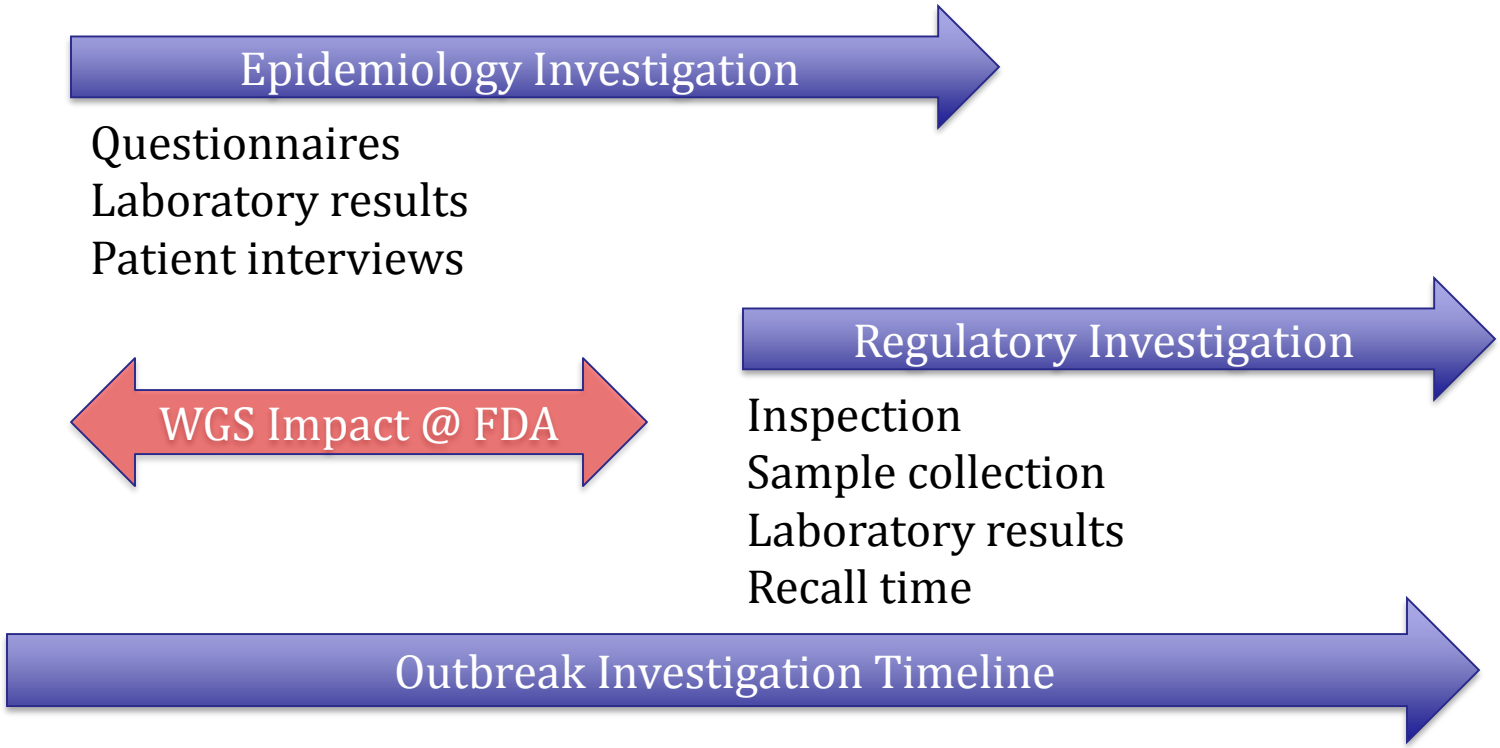
The U.S. Food and Drug Administration (FDA) hereby issues this Order to suspend the registration of your food facility, Roos Foods, Inc. (Roos), located at 251 Roos Lane, Kenton, DE 19955. Your food facility was registered with FDA pursuant to section 415(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 350d(a)) on June 4, 2013. Section 415(b)(1) of the FD&C Act provides, in relevant part, that if FDA determines that a food manufactured, processed, packed, received, or held by a facility registered under section 415 has a reasonable probability of causing serious adverse health consequences or death to humans or animals, FDA may by order suspend the registration of a facility (1) that created, caused, or was otherwise responsible for such reasonable probability; or (2) that knew of, or had reason to know of, such reasonable probability, and packed, received, or held such food.

S. Braenderup





Outbreak Investigation Timelines



Immediate impacts of WGS to industry, growers, and distributers, countries, states.

- Earlier intervention means:
  - Reduced amount of recalled product
  - Fewer sick patients which means fewer lawsuits
  - Less impact overall and minimal damage to brand recognition

FDA WGS Application to Actual Food Contamination Events

Montevideo black and red pepper
Senftenberg black and red pepper
Enteritidis shell/liquid eggs
Heidelberg ground turkey
Heidelberg chicken broilers
Heidelberg chicken livers
Enteritidis custard
Bareilly tuna scrape
Tennessee peanut butter/peanut butter paste
Typhimurium peanut butter
Braenderup peanut butter/nut butter
Tennessee cilantro
Agona dry cereal
Agona papaya
Newport tomatoes
Newport environmental
Kentucky - Cerro dairy/dairy farms
Anatum spices/pepper flakes
Javiana cantaloupes
Saintpaul hot peppers
4,5,12: i –
Javiana/Newport Cucumbers
Montevideo Pistachios
Hartford Chia powder
Mbandaka Tahini Sesame paste
Braenderup Mangoes
Poona Cucumbers

Lmono cantaloupes
Lmono queso cheese
Lmono potato salad
Lmono artisanal cheeses
Lmono avocados
Lmono ricotta
Lmono celery/chix salad
Lmono smoked fish
Lmono other herbs
Lmono peaches
Lmono hot peppers
Lmono tofu
Lmono sprouts
Lmono ice cream

Cronobacter infant formula
V para oysters
EcO157:H7 lettuce
STEC beef
STEC flour

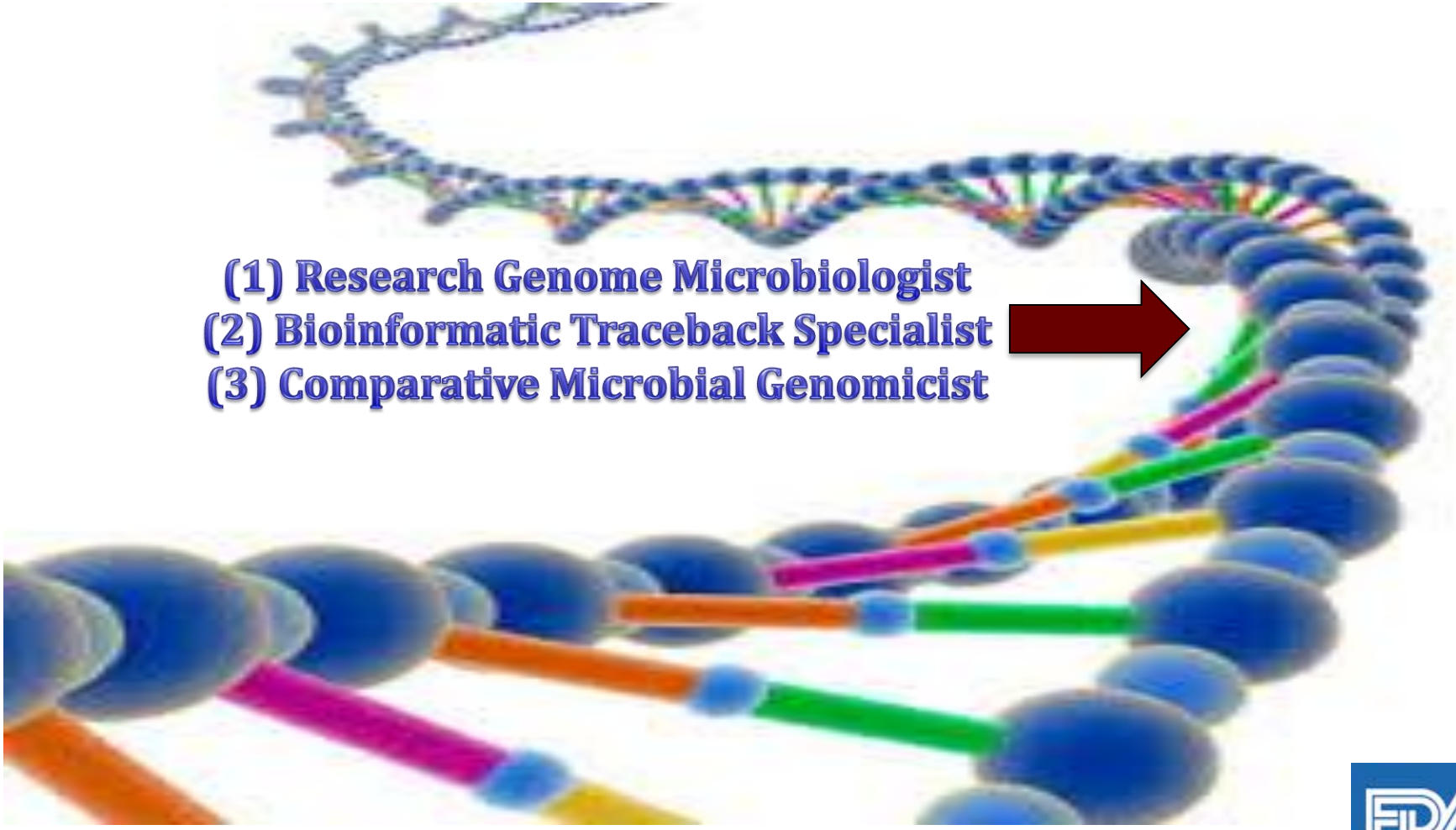
Applications of WGS in the Food Safety Environment

- Delimiting scope and traceback of food contamination events (Track-N-Trace)
- Quality control for FDA testing and surveillance (Confidence in Regulatory Actions)
- Preventive control monitoring for compliance standards (the “repeat offender” project)
- ID, geno/pheno typing schemes (AST,Serotyping, VP) (CVM,CDRH,CFSAN) – risk assessment and adaptive change in *Salmonella* and *Listeria*

Some Recent Metrics….

1. Number of FDA GenomeTrakr laboratories operational.	13
2. Number of Federal GenomeTrakr laboratories operational (FSIS and other partners).	2
3. Number of State GenomeTrakr laboratories operational.	25
4. Number of International GenomeTrakr laboratories operational.	20
5. Number of IFSH Industry WGS Workgroup Members	4
6. Number of isolates sequenced by Genome-Trakr Network Number of *Salmonella* isolates sequenced over time by the GenomeTrackr Network.	64357
b. Number of *Listeria* isolates sequenced over time by the GenomeTrackr Network.	13470
c. Number of *Campylobacter* isolates sequenced over time by the GenomeTrackr Network.	7923
d. Number of *Cronobacter* isolates sequenced over time by the GenomeTrackr Network.	58
e. Number of *E. coli/Shigella* isolates sequenced over time by the GenomeTrackr Network.	26891
f. Cumulative number of compliance actions/cases supported by WGS (in conjunction with OAO)	196
g. Cumulative number of sequences generated from archived collections of isolates in Office of Regulatory Affairs to enhance compliance and enforcement databases.	6394

WGS is Spawning New Careers in Microbiology !!!!!



(1) Research Genome Microbiologist
(2) Bioinformatic Traceback Specialist
(3) Comparative Microbial Genomicist

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**
  - Eastern Laboratory
- **CDC**
  - Enteric Diseases Laboratory
- **INEI-ANLIS "Carolos Malbran Institute," Argentina**
- **Centre for Food Safety, University College Dublin, Ireland**
- **Food Environmental Research Agency, UK**
- **Public Health England, UK**
- **WHO**
- **Illumina**
- **Pac Bio**
- **CLC Bio**
- **Dr. Lourdes Simental and Other independent collaborators**