



16th Annual PulseNet Meeting, Atlanta, GA
August 29, 2012



“Use of Next-Generation Sequencing to Augment Characterization of *Salmonella enterica* strains Associated with Contamination of the Food Supply ”

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“Cool...Now what?”

“So, I’ ve sequenced 4 million bp of DNA for 106 *Salmonella* strains”

“Umm, I’ ve sequenced 4 million bp of DNA for 106 *Salmonella* strains...”



FDA circa 1906

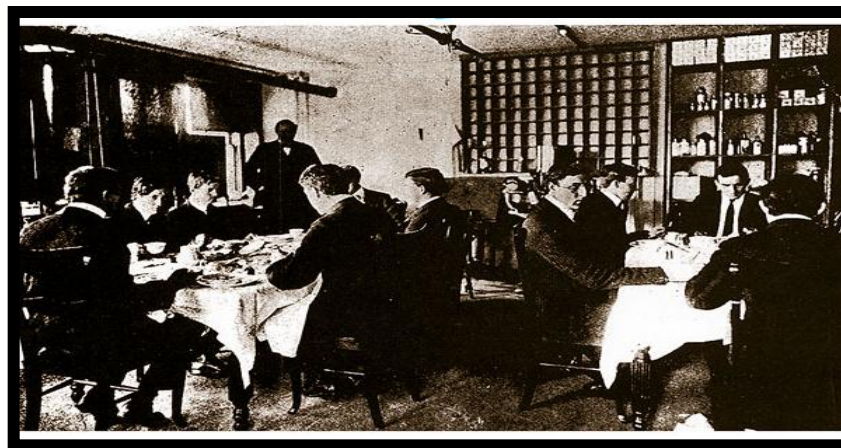
PRESENTATION OBJECTIVES

(1) To frame the importance and rationale for application of next-generation typing methods in food safety, including NGS.

(2) To identify specific areas and situations where current NGS approaches can augment the laboratory's contribution to food outbreak investigations.

(3) To present several retrospective examples where application of NGS provided additional insight for (i) delimiting the scope of a contamination event; (ii) affirming a cluster from diverged background genotypes; and (iii) source-tracking by comparative genomics of food and environmental isolates of *Salmonella*.

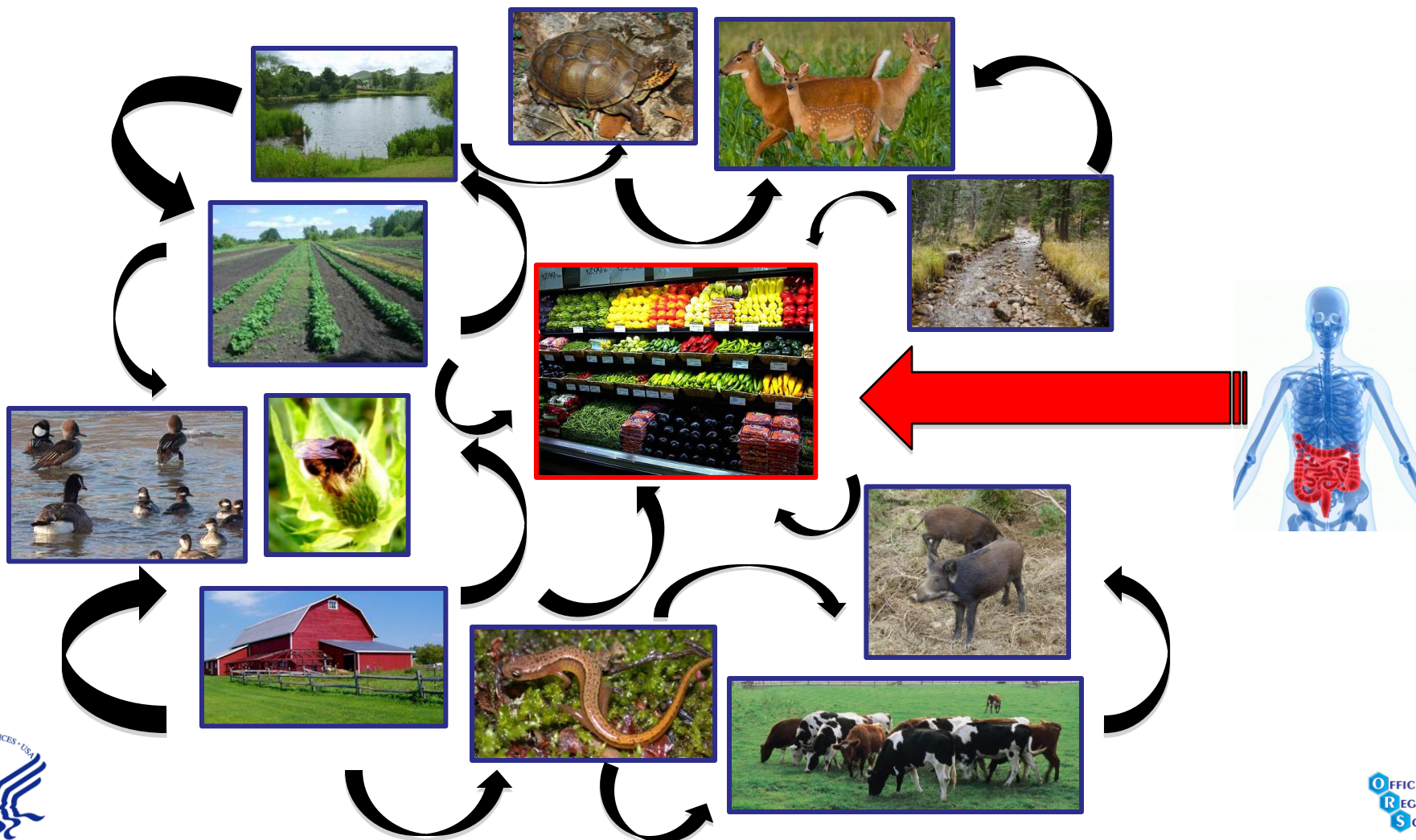
(4) To discuss challenges associated with next-next generation sequencing and the pursuit of characterization of foodborne pathogens directly from foods.



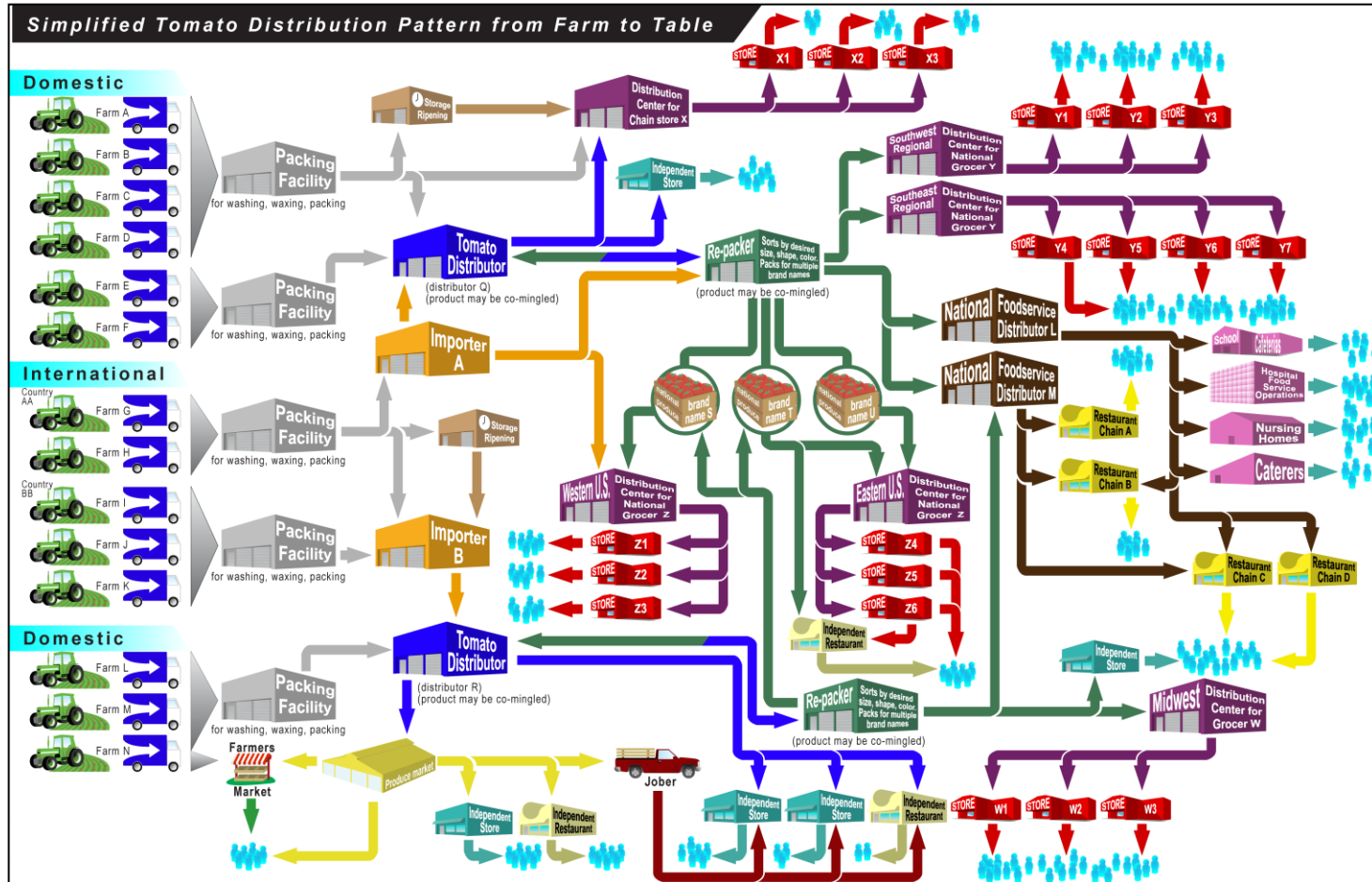
***Salmonellae are emerging
in a wide variety of foodborne niches:***



...And are difficult to traceback due to complex disseminations on the farm



...and that is just on the farm. Once it reaches the supply chain, things really “simplify”.



The Fresh-cut Tomato Supply Chain



“The Smokin’ Hot Pepper”

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FOOD SAFETY

Salmonella 'smoking gun' located

July 31, 2008 | Tiffany Hsu | Times Staff Writer

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A strain of the salmonella bacteria that sickened more than 1,300 people has been found in a serrano pepper and a sample of irrigation water at a farm in Mexico, U.S. health officials said Wednesday.

They called the discovery a "breakthrough" but cautioned that tomatoes may still be a culprit in the nearly four-month outbreak that has alarmed consumers and cost the domestic produce industry hundreds of millions of dollars.

(Salmonella Saintpaul outbreak – Summer 2008)





**FDA Foods and Veterinary Medicine Program
Strategic Plan (2012 – 2016)
OF/CFSAN/CVM/ORR/NCTR**

**Program Goal 6: Improve detection of and
response to foodborne outbreaks and
contamination incidents**

Obj. 1: Improve response to foodborne outbreaks and
contamination incidents

Obj. 2: Investigate and adopt innovative technologies
and processes to detect and investigate foodborne
outbreaks and contamination events

?

?

Next-Generation Sequencing

A high-throughput sequencing method that parallelize the sequencing process, producing thousands or millions of sequences at once.

Over the past five years, so called “Next-Generation” sequencing technologies have made accessible data capable of answering questions fundamental to our understanding of life and the factors that govern human health. The combination of the vast increase in data generated, coupled with plummeting costs required to generate these data, has rendered this technology a tractable, general purpose tool for a variety of applications.

?



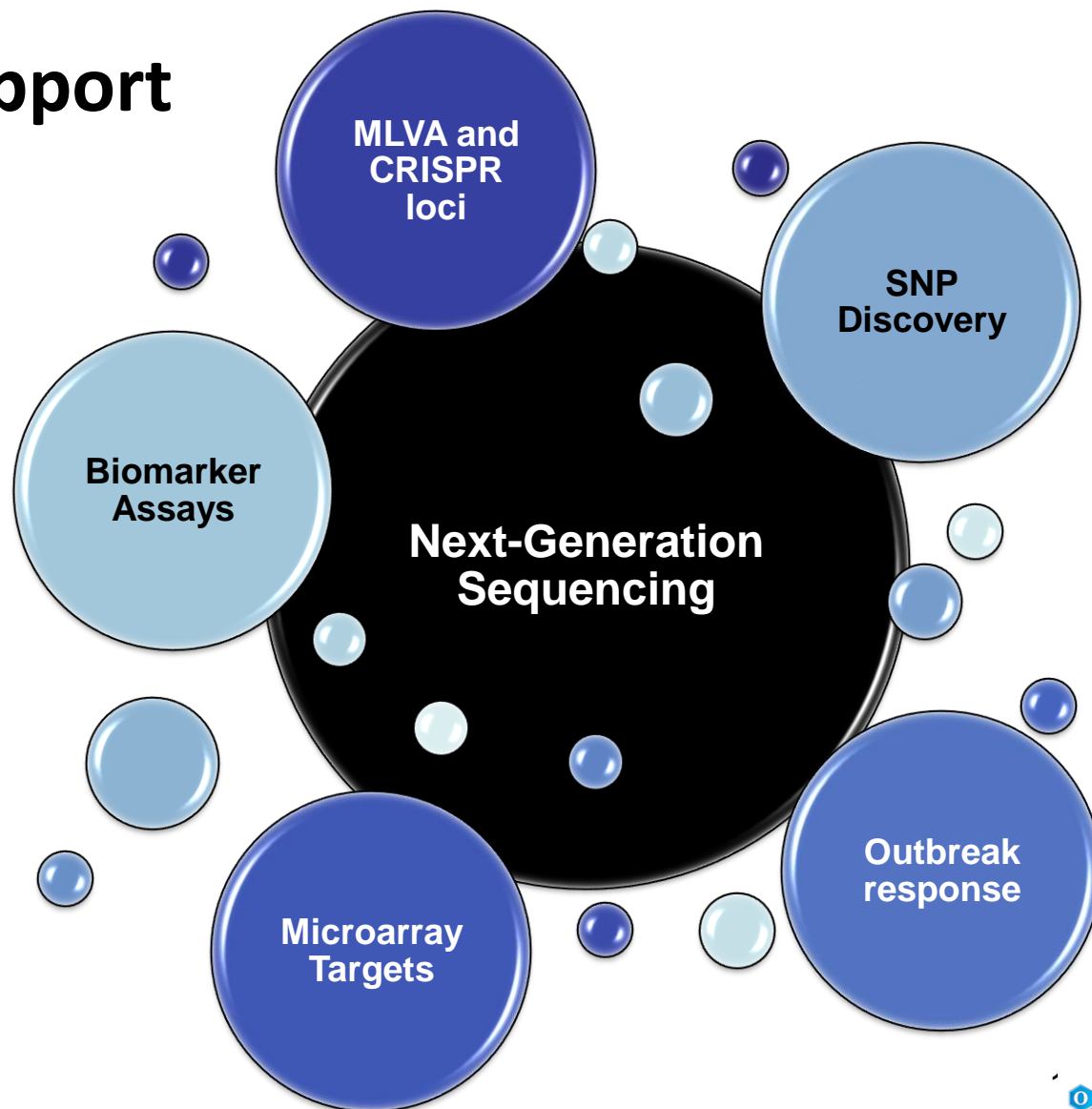
?



NGS Provides support for other technologies



Next-Generation Sequencing (NGS)



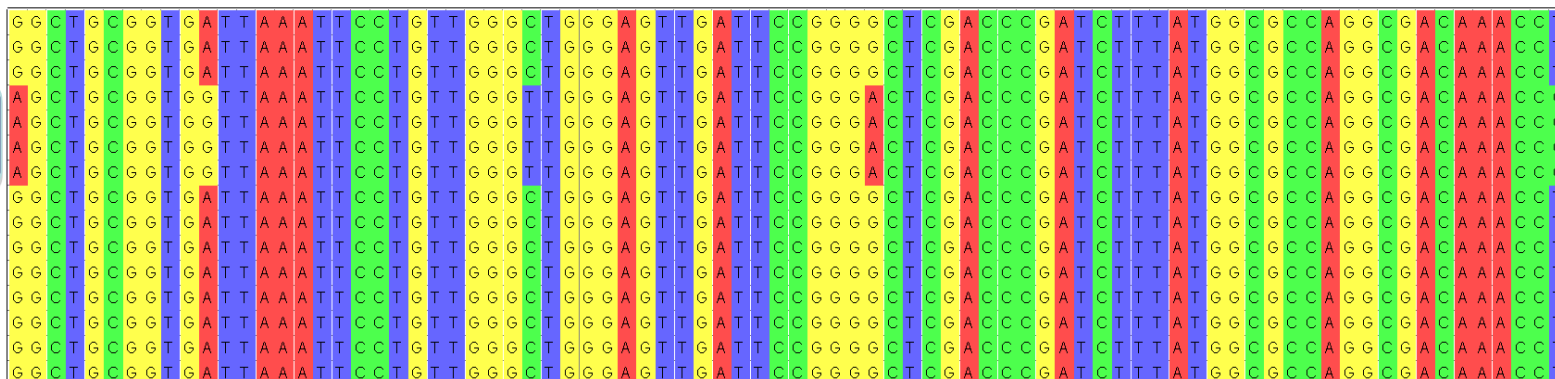


Multiple Sequence Alignment Places the Individual Changes across a DNA Sequence into Their Proper Data Bins

Taxon \ Character
 SEEM965_human
 SEEM675_human
 SEEM054_76_39|human

Non-outbreak

SEEM202_06293|SEEM202|spice
 SEEM201_20876|SEEM201|spice
 SEEM971_16932|SEEM971|spice
 SEEM973_03847|SEEM973|spice
 SEEM974_01761|SEEM974|spice
 SEEM315_17925|SEEM315|pistachio
 SEEM316_05086|SEEM316|pistachio
 SEEM954_07553|SEEM954|pistachio



The trick is doing it for 4 million bp across 100 different strains
at the same time.



➤ N Engl J Med. 2011 Feb 24;364(8):730-9. [Whole-genome sequencing and social-network analysis of a tuberculosis outbreak](#). Gardy JL, Johnston JC, Ho Sui SJ, Cook VJ, Shah L, Brodtkin E, Rempel S, Moore R, Zhao Y, Holt R, Varhol R, Birol I, Lem M, Sharma MK, Elwood K, Jones SJ, Brinkman FS, Brunham RC, Tang P. British Columbia Centre for Disease Control, Vancouver, BC, Canada.

➤ Science. 2010 Jan 22;327(5964):469-74. [Evolution of MRSA during hospital transmission and intercontinental spread](#). Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD. The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

➤ N Engl J Med. 2011 Jan 6;364(1):33-42. Epub 2010 Dec 9. [The origin of the Haitian cholera outbreak strain](#). Chin CS, Sorenson J, Harris JB, Robins WP, Charles RC, Jean-Charles RR, Bullard J, Webster DR, Kasarskis A, Peluso P, Paxinos EE, Yamaichi Y, Calderwood SB, Mekalanos JJ, Schadt EE, Waldor MK. Pacific Biosciences, Menlo Park, CA, USA.

➤ Science. 2011 Jan 28;331(6016):430-4. [Rapid pneumococcal evolution in response to clinical interventions](#). Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, McGee L, von Gottberg A, Song JH, Ko KS, Pichon B, Baker S, Parry CM, Lambertsen LM, Shahinas D, Pillai DR, Mitchell TJ, Dougan G, Tomasz A, Klugman KP, Parkhill J, Hanage WP, Bentley SD. The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

➤ Proc Natl Acad Sci U S A. 2010 Apr 20;107(16):7527-32. Epub 2010 Apr 5. [Evolutionary dynamics of Clostridium difficile over short and long time scales](#). He M, Sebahia M, Lawley TD, Stabler RA, Dawson LF, Martin MJ, Holt KE, Seth-Smith HM, Quail MA, Rance R, Brooks K, Churcher C, Harris D, Bentley SD, Burrows C, Clark L, Corton C, Murray V, Rose G, Thurston S, van Tonder A, Walker D, Wren BW, Dougan G, Parkhill J. Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA, United Kingdom.

➤ N Engl J Med. 2011 Mar 10;364(10):981-2. Epub 2011 Feb 23. [Identification of a salmonellosis outbreak by means of molecular sequencing](#). Lienau EK, Strain E, Wang C, Zheng J, Ottesen AR, Keys CE, Hammack TS, Musser SM, Brown EW, Allard MW, Cao G, Meng J, Stones R.. FDA, College Park, MD, USA

➤ [Rise of the Superbacteria](#) - The O104:H4 genome sequence suggests that it's yet another concoction from evolution's cauldron. The bacteria contain many segments of DNA not seen in other E. coli strains. This new DNA may be responsible for its high level of virulence—more - Newsweek · 6/5/2011

 Sci Transl Med 22 August 2012. DOI: 10.1126/scitranslmed.3004129

[Tracking a Hospital Outbreak of Carbapenem-Resistant Klebsiella pneumoniae with Whole-Genome Sequencing](#)

Evan S. Snitkin¹, and others and Julia A. Segre^{1,*}, National Human Genome Research Institute, Bethesda, MD 20892, USA.

National Institutes of Health Clinical Center, Bethesda, MD 20892, USA.

WGS-based characterization of salmonellae associated with the food supply

Many of our projects fall into one of two categories that challenge PFGE:

Same PFGE Pattern (but we suspect that not everything is related)

Different PFGE Patterns (but we suspect they may be related)



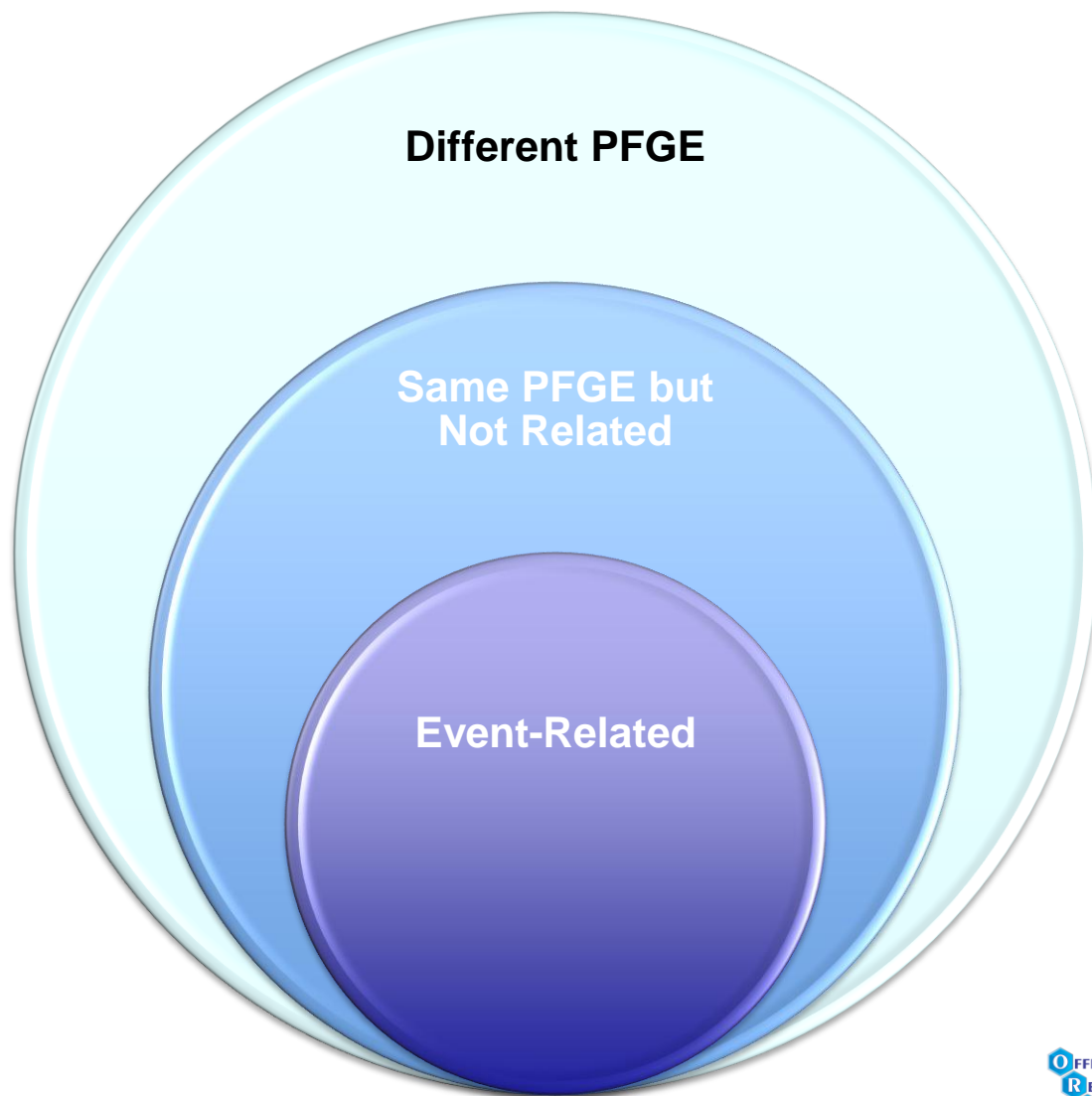
This region contains isolates where we are comfortable that PFGE will differentiate unrelated isolates

General NGS Questions that Can be Asked:

1. Is a particular isolate part of the outbreak?
 - Or is it background?
2. Have we seen this isolate before?
 - i.e.) seasonality in S. Newport from Eastern Shore
3. Does this food/environmental isolate match this clinical isolate?
 - Can we link an isolate from food or manufacturing facility to an outbreak?

NGS Analysis Strategy

- We try to include several sets of isolates to ensure that NGS can answer the questions that we're asking for this organism and/or serotype

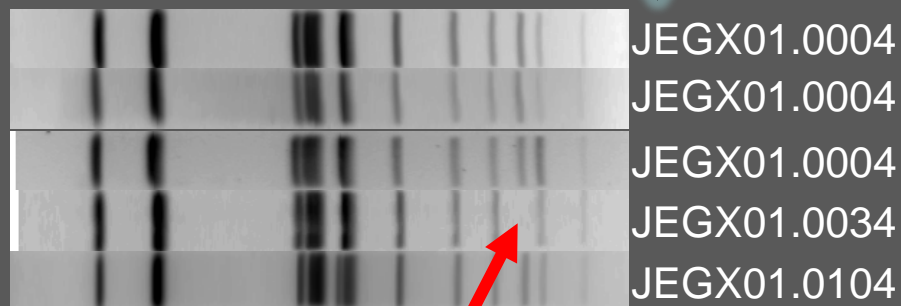


Same PFGE Pattern

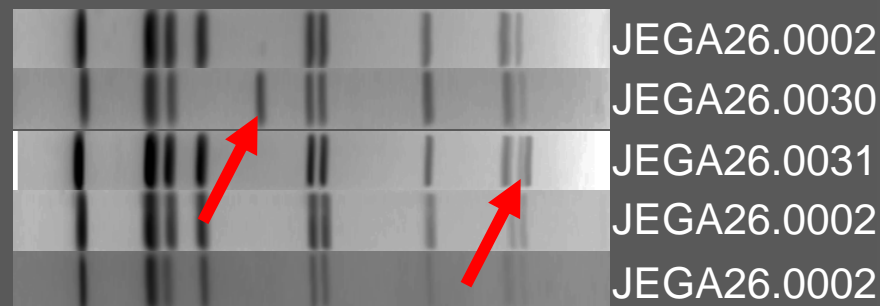
S. Enteritidis

Several pattern combinations were found during the 2010 egg outbreak, but JEGX01.0004 is 40% of all of the S. Enteritidis seen in the PulseNet database

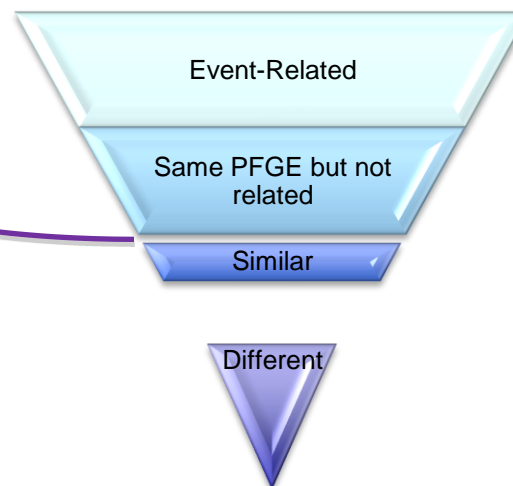
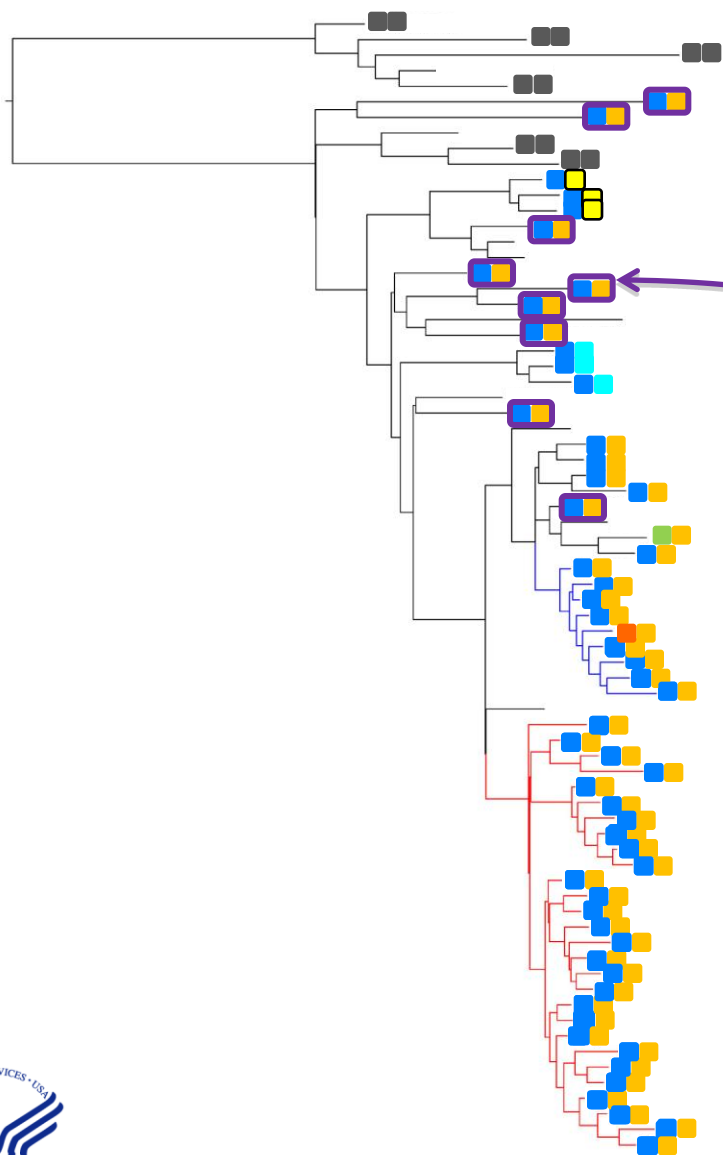
*Xba*I – Primary Enzyme



*Bln*I – Secondary Enzyme



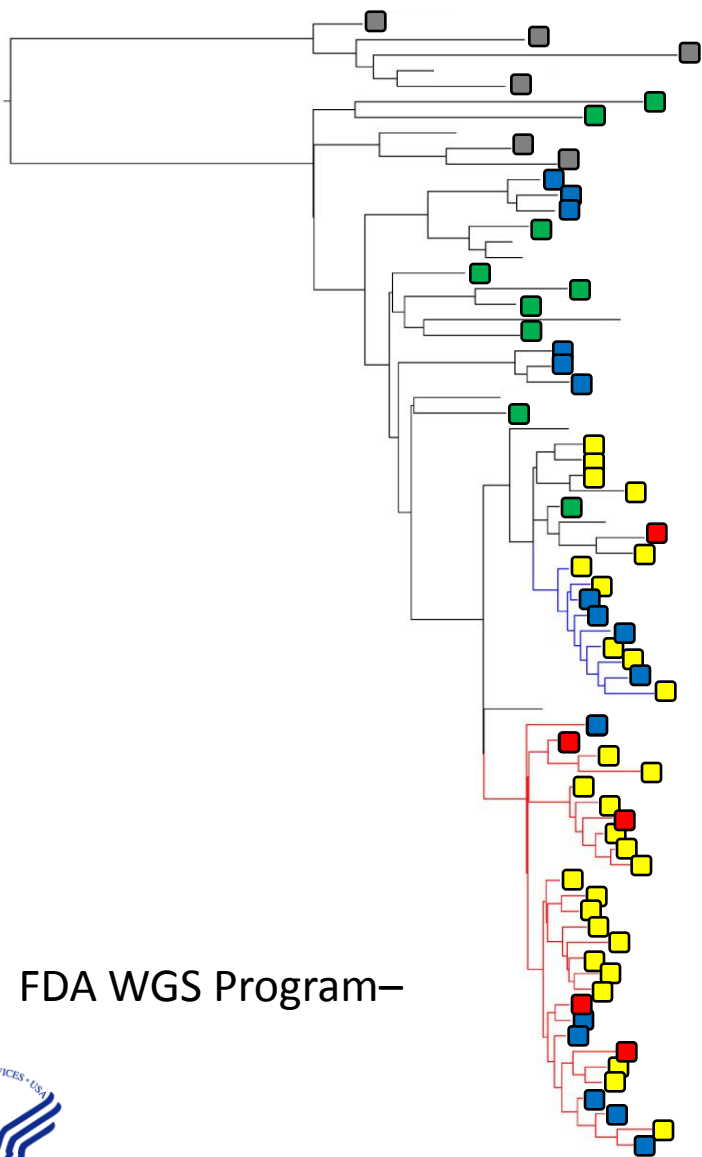
S. Enteritidis 2010 Shell egg Outbreak



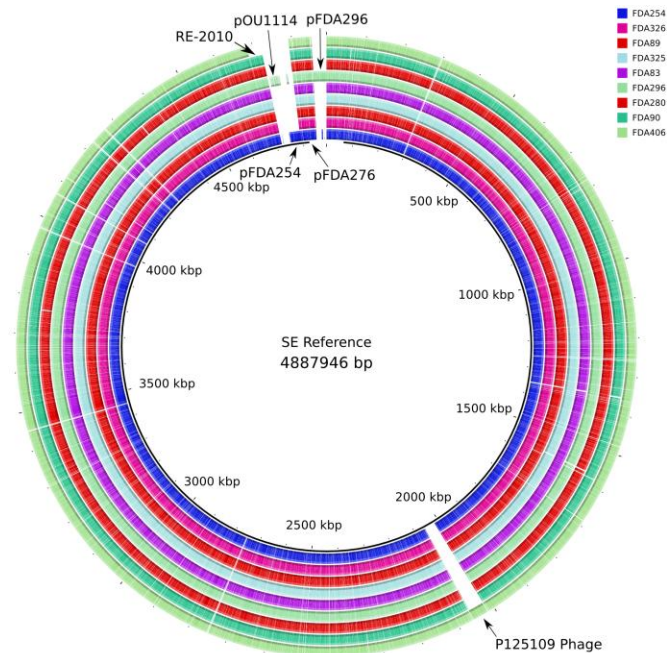
XbaI Patterns	BlnI Patterns
JEGX01.0004	JEGA26.0002
JEGX01.0034	JEGX01.0030
JNXX01.0104	JNXX01.0031
Very Different	

S. Enteritidis

- Unrelated Reference Isolates – different PFGE
- Unrelated Reference Isolates – same PFGE
- Clinical Isolates
- Egg or Related Food Isolates
- Environmental Isolates



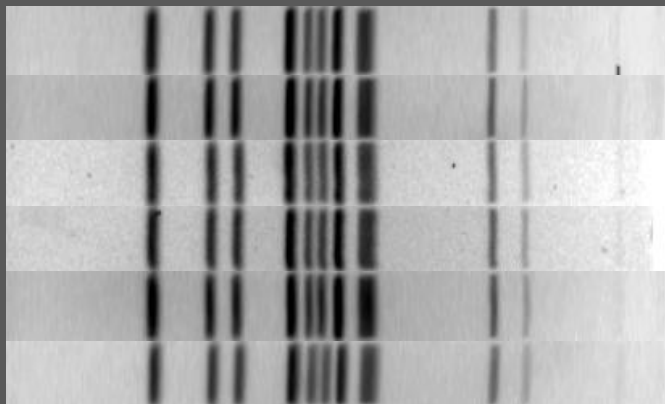
FDA WGS Program—



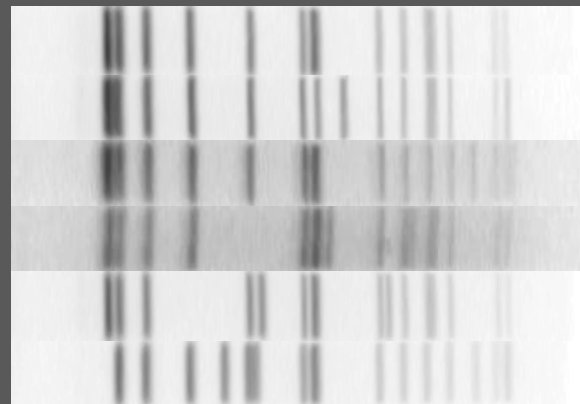
Different PFGE Patterns

Salmonella Agona 1998/2008 Dry Cereal Outbreaks

*Xba*I – Primary Enzyme

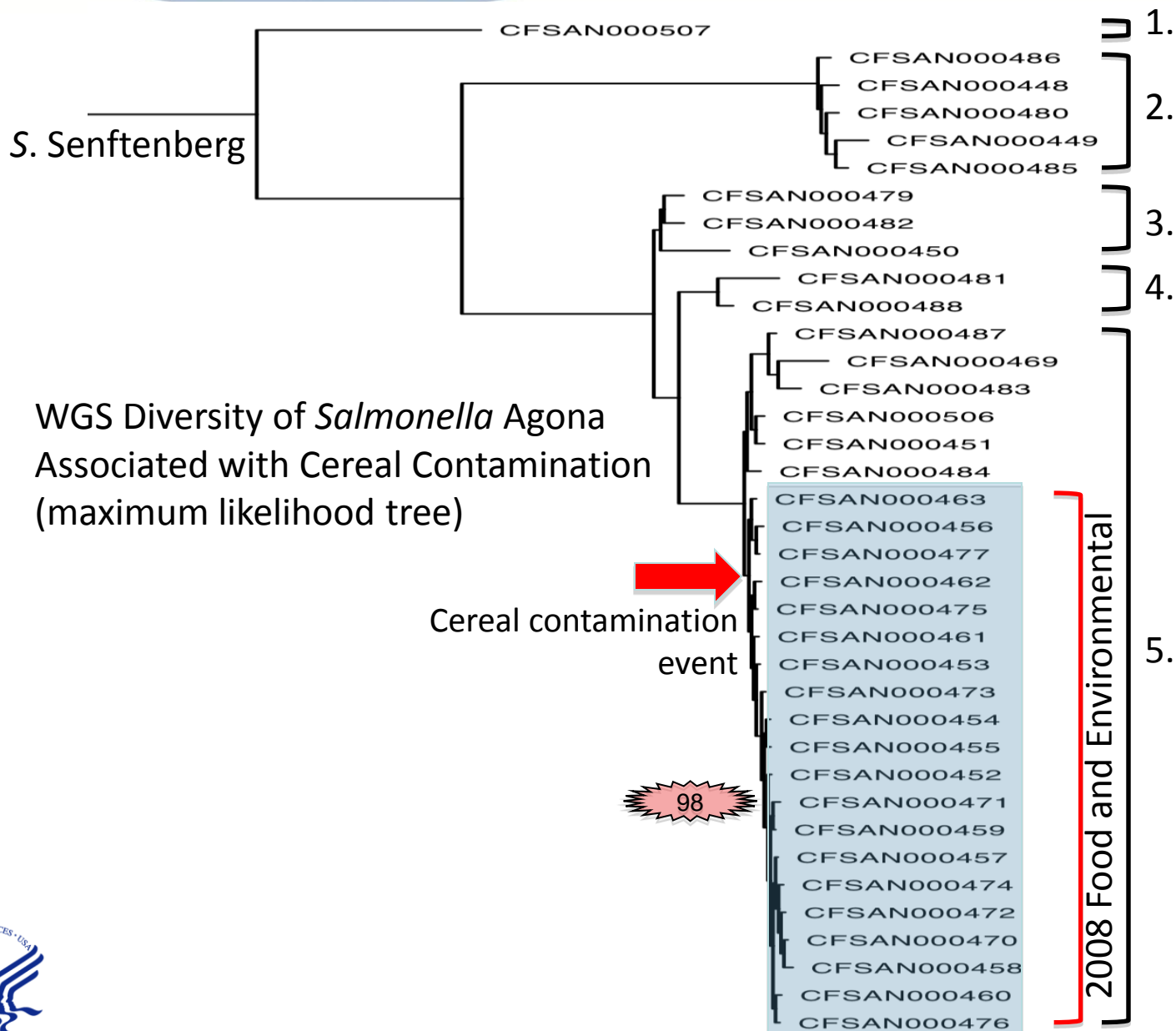


*Bln*I – Secondary Enzyme



Food	2008
Food	1998
Env.	2008
Env.	2008
Food	2008
Env.	2008

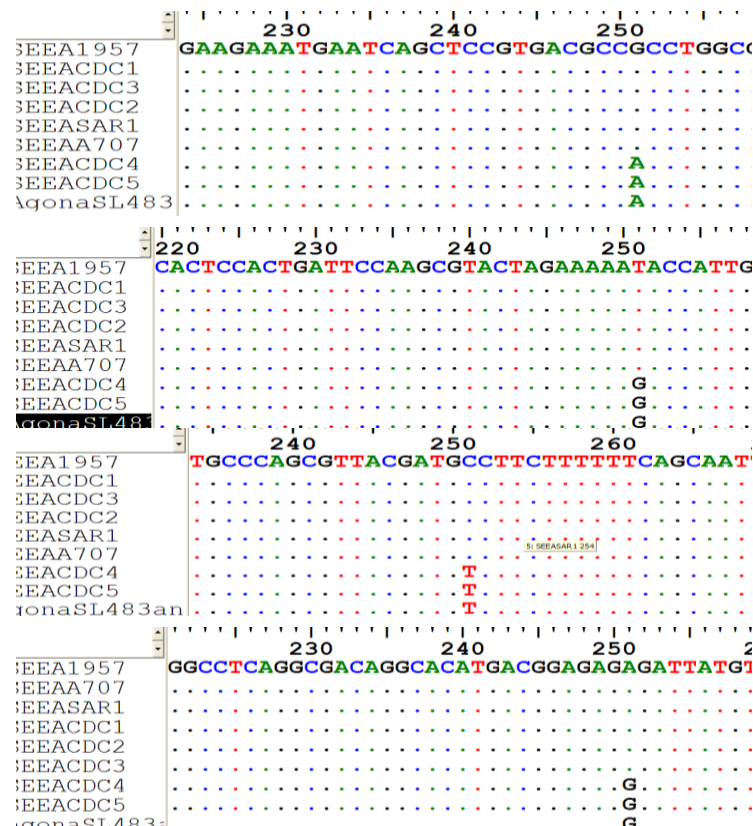
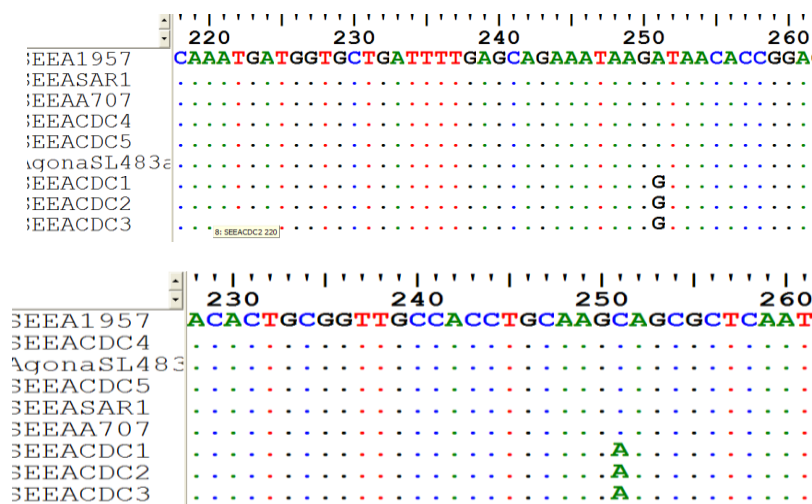
S. Agona clade:



SNP variation among *S. Agona* over 10 years

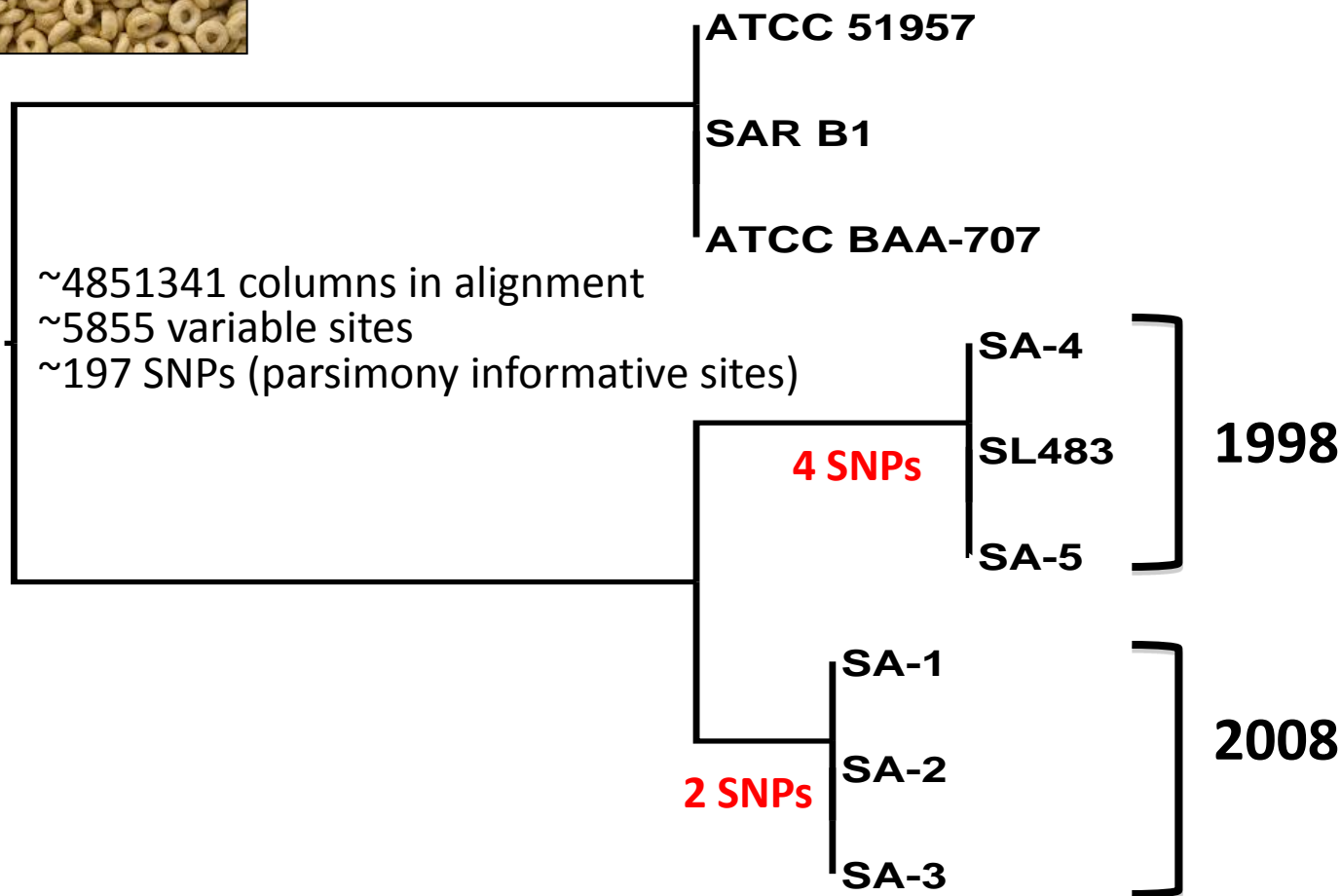
SA-1, SA-2, SA-3

SA-4, SA-5, SL483





Clustering of filtered SNPs delineates 10 year divergence of *S. Agona* cereal isolate JABX01.0001



***Salmonella* Agona Dry Cereal Outbreak**



Salmonella Agona strains could be readily distinguished based on WGS analysis – providing a set of 5 unique and stable genetic differences that defined the two temporally related isolate groups underscoring the ability to differentiate and track an isolate over time within the same facility.



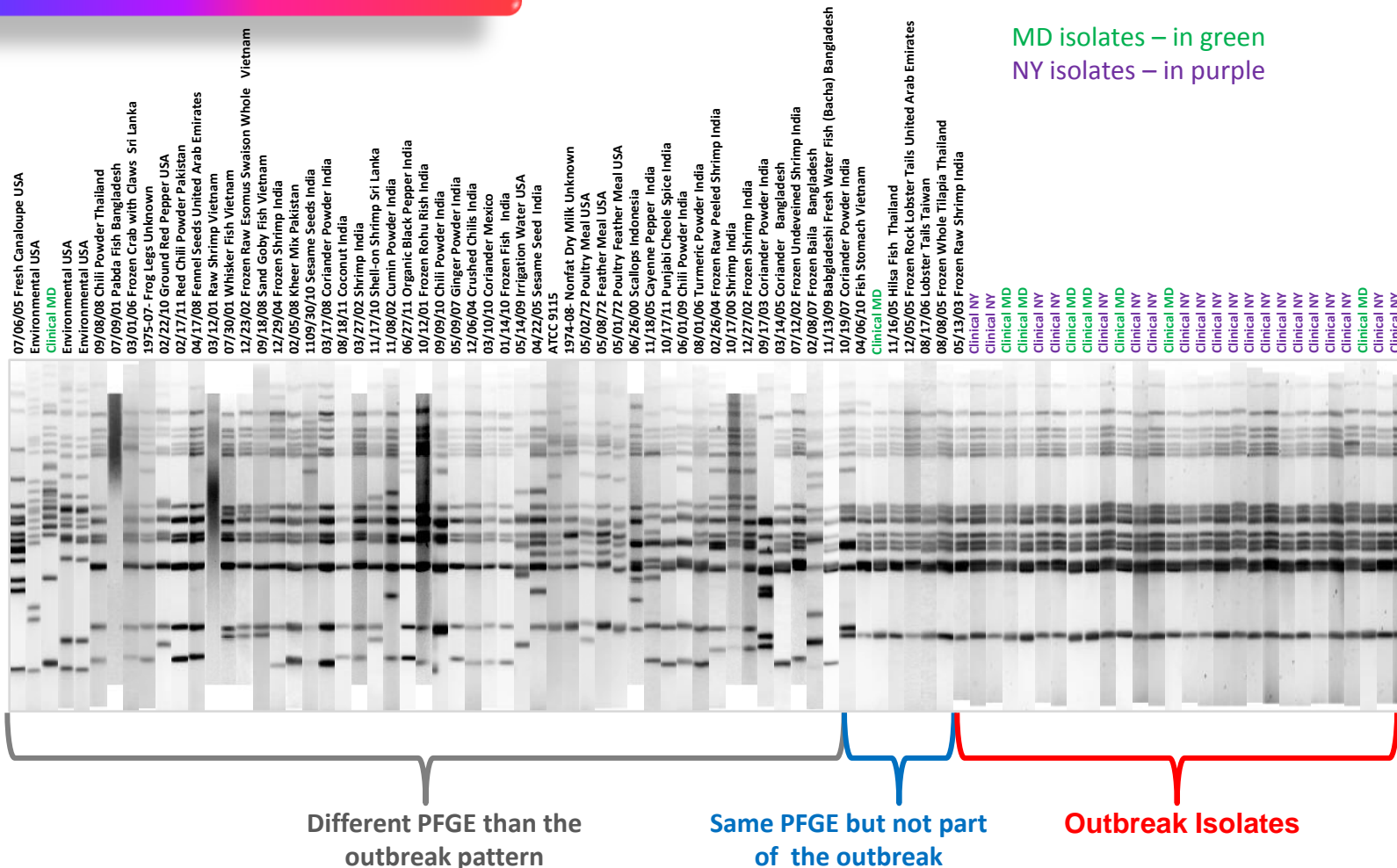
The re-emergence of the isolate in the facility may have been a combination of renovations in an older side of the plant which included drilling into a wall, previously well-sanitized, which could have released the dormant pathogen; also, wet cleaning sanitation practices could have spread the pathogen throughout that part of the facility.



Training of facility employees was re-emphasized to reduce risk of spreading *Salmonella* as was use of dry sanitation over wet cleaning methods.

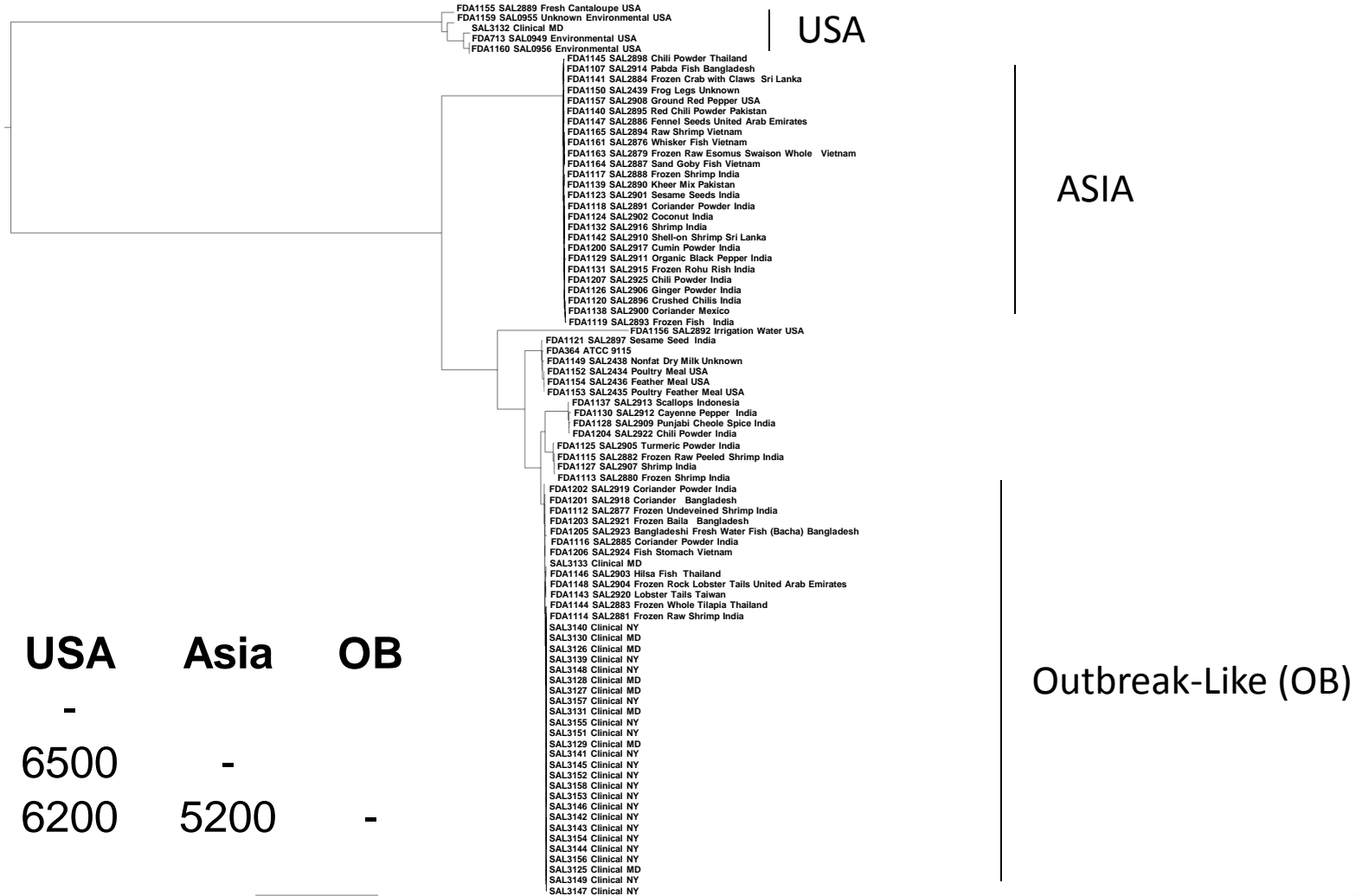
S. Bareilly PFGE

Source Tracking





S. Bareilly Phylogeny

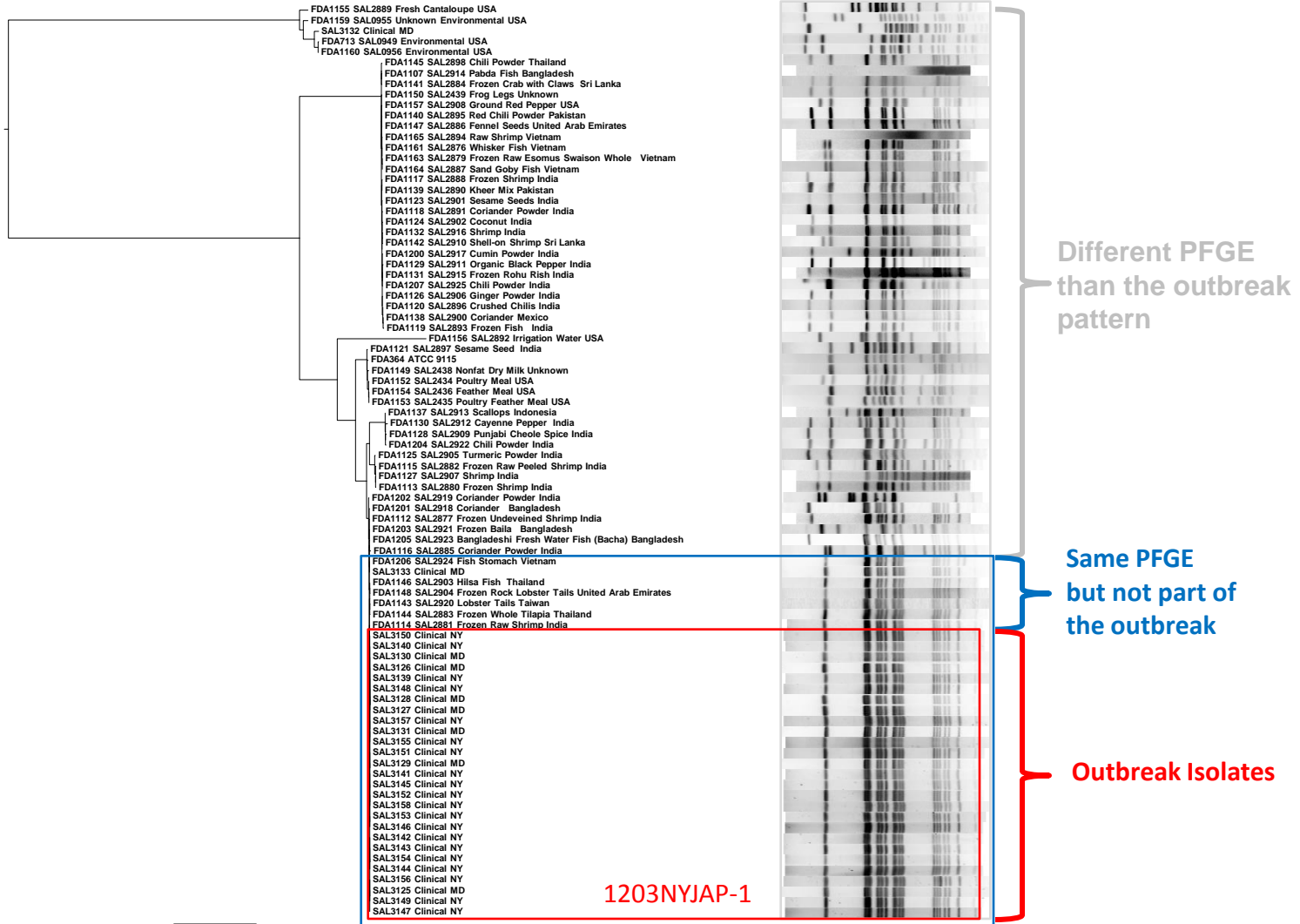


	USA	Asia	OB
USA	-		
Asia	6500	-	
OB	6200	5200	-

Outbreak-Like (OB)

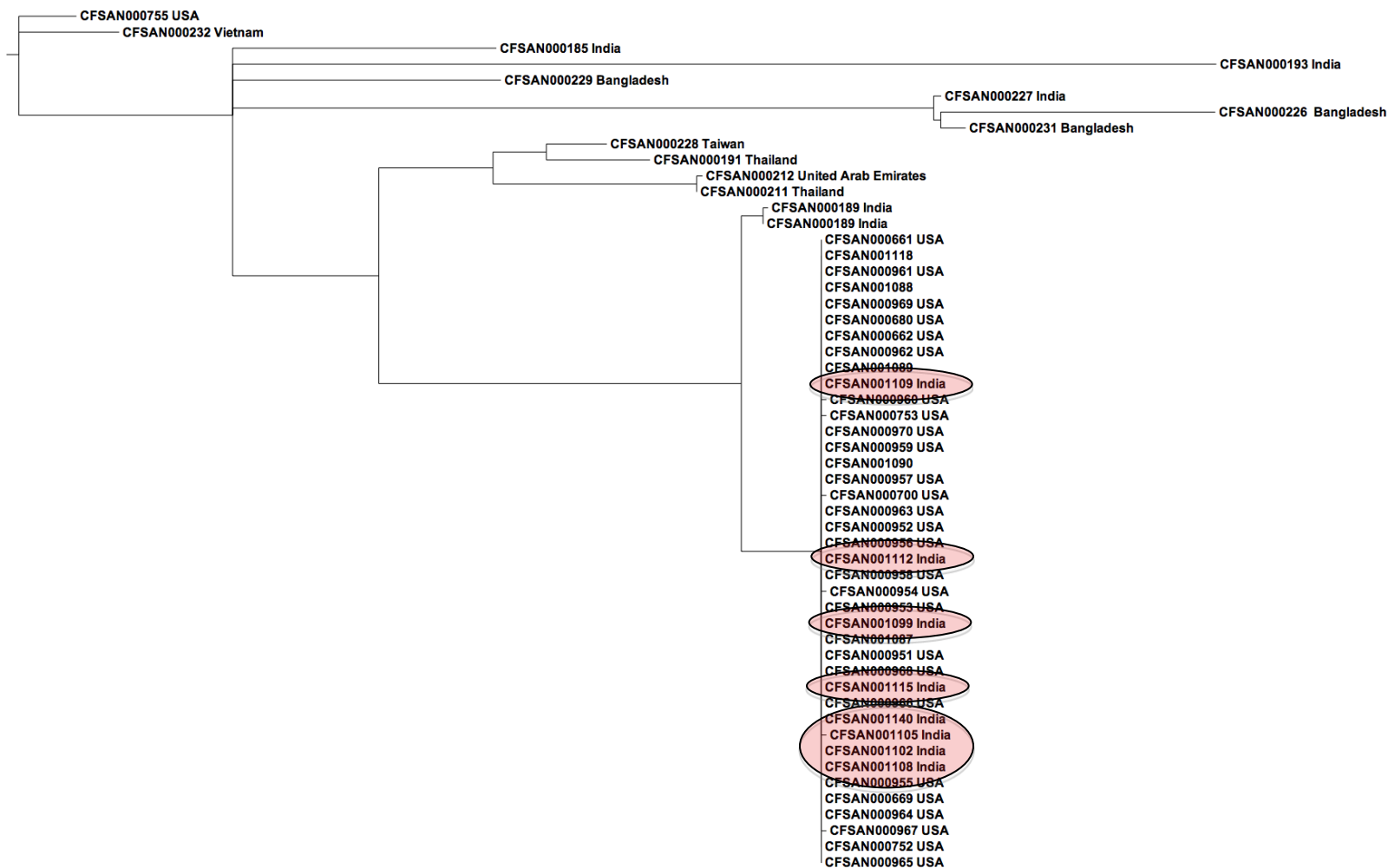


S. Bareilly Phylogeny & PFGE





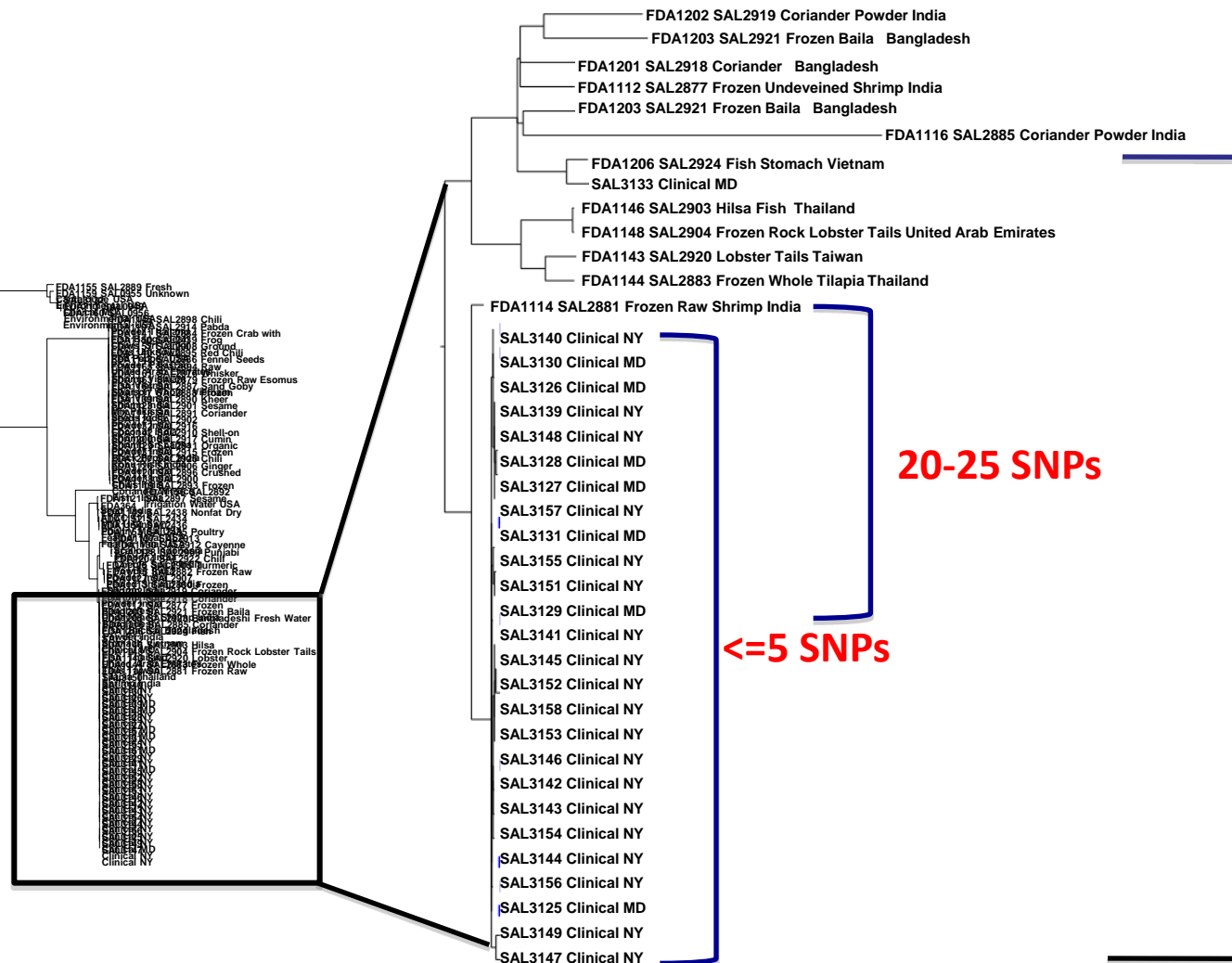
S. Bareilly from Tuna Scrape



0.04

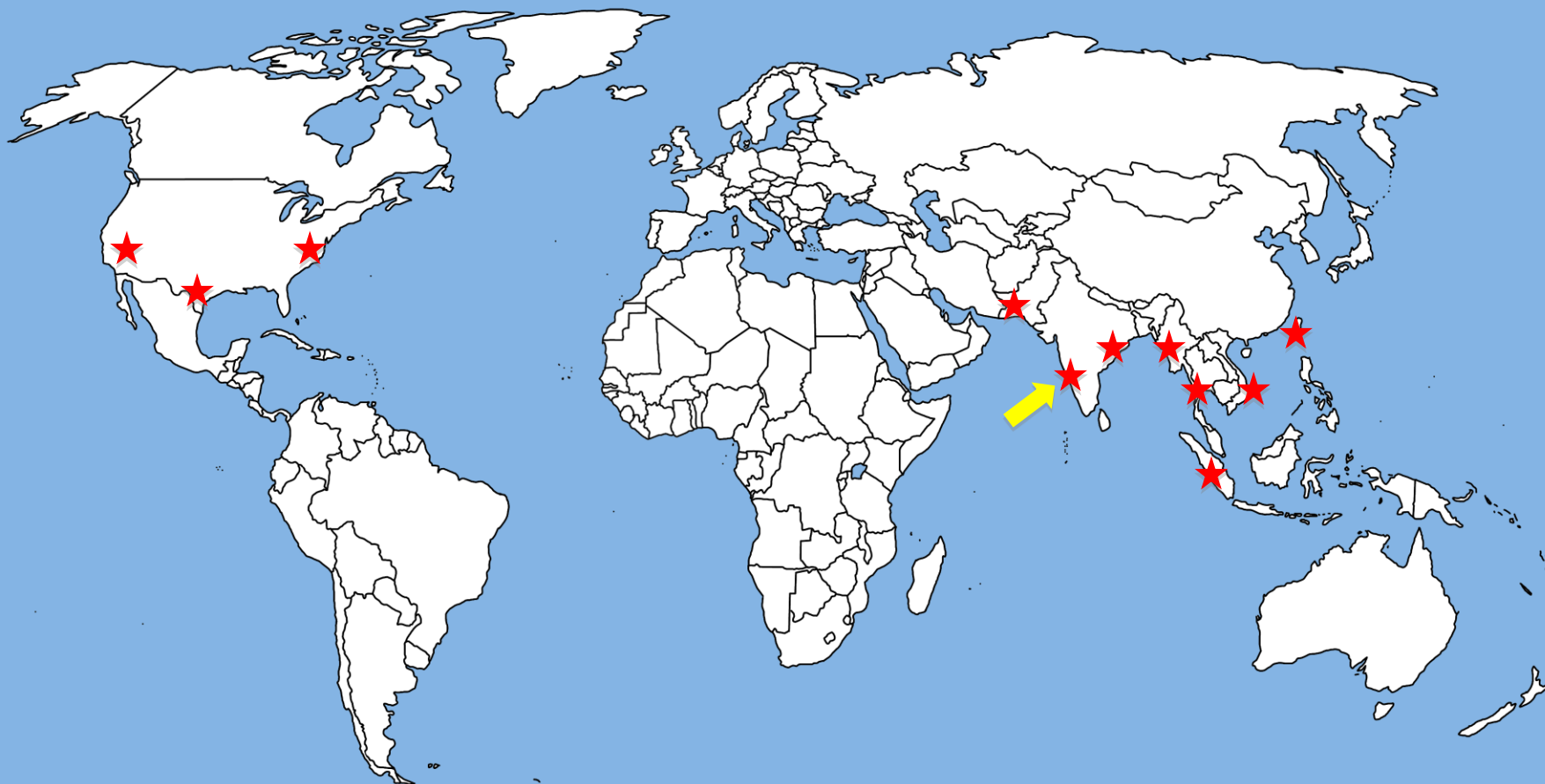


S. Bareilly Phylogeny



5.0E-4

NGS distinguishes geographical structure among closely related *Salmonella* Bareilly



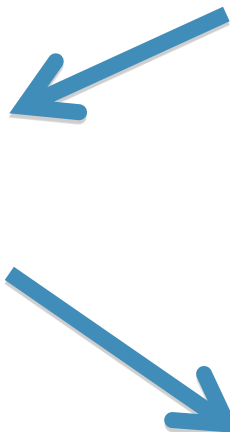
The 100K Pathogen Genome Project

The “100K Pathogen Genome Project” is a public database of food pathogen genomes that will provide a roadmap for public-private development of methods to aid in quick identification and tracking of pathogens back to their source.

UCDAVIS

100K Genome Project

- **Consortium**
 - Sequencing to be done at BGI@UCDavis
 - Initial ~500 genomes will be closed
 - Genomes will be placed in the public domain
 - Metadata submission
- **Additional partnerships**
 - Unique isolates
 - World wide representation
 - Food industry
 - Government
 - Academia
- **Outcomes**
 - Population based assessment for new assay design
 - Clinical vs food isolates
 - Outbreak and trace back
 - Examine biogeography of genome to focus
 - SNPs for local divergence
 - Virulence and AR
 - An entire collection of isolates that match genomes



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100K Pathogen Genome Project

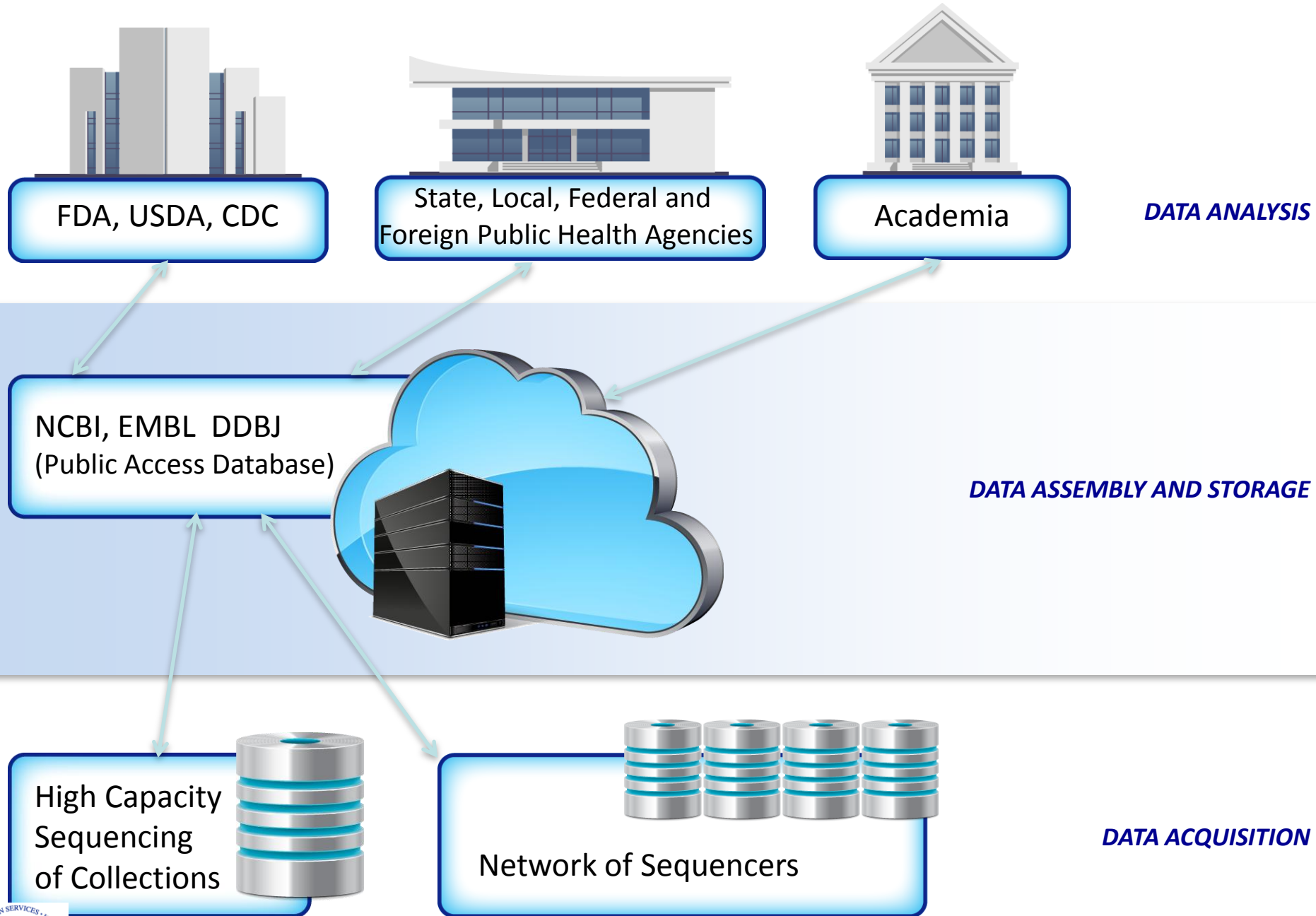
Bart Weimer, Ph.D.
Co-Director, BGI@UCDavis
Professor, School of Veterinary Medicine

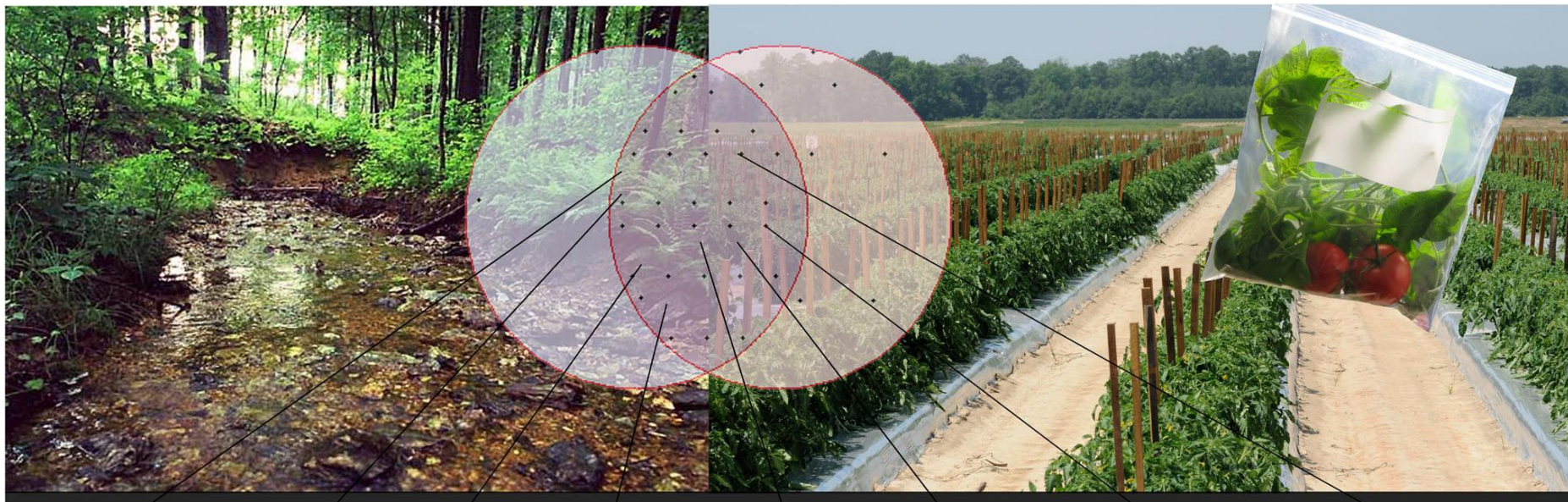
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Organisms of Interest

- *Salmonella*
- *E. coli*
- *Listeria*
- *Campylobacter*
- *Vibrio*
- *Shigella*
- *Yersinia*
- *Clostridium*
- *Enterococcus*
- *Cronobacter*
- **Norovirus**
- **Hepatitis A**

More information:
BCWEIMER@UCDAVIS.EDU





Ciona



Danio



Drosophila



Crematogaster



Gallus



Culex



Bemisia



Xenopus



Fugu



Apis



Peristenus



Melanophthalma



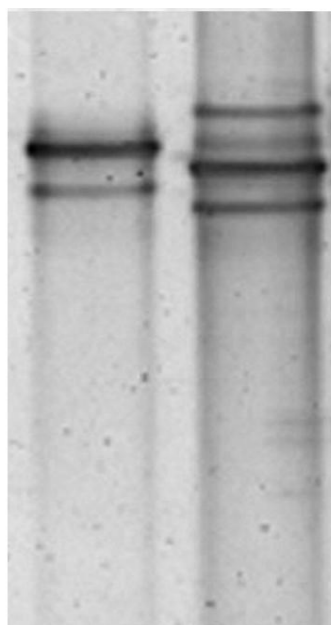
Eccoptura



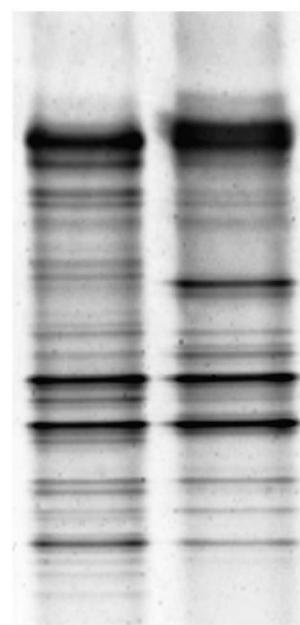
Elaphrus

Metagenomics

Cultured vs. Uncultured

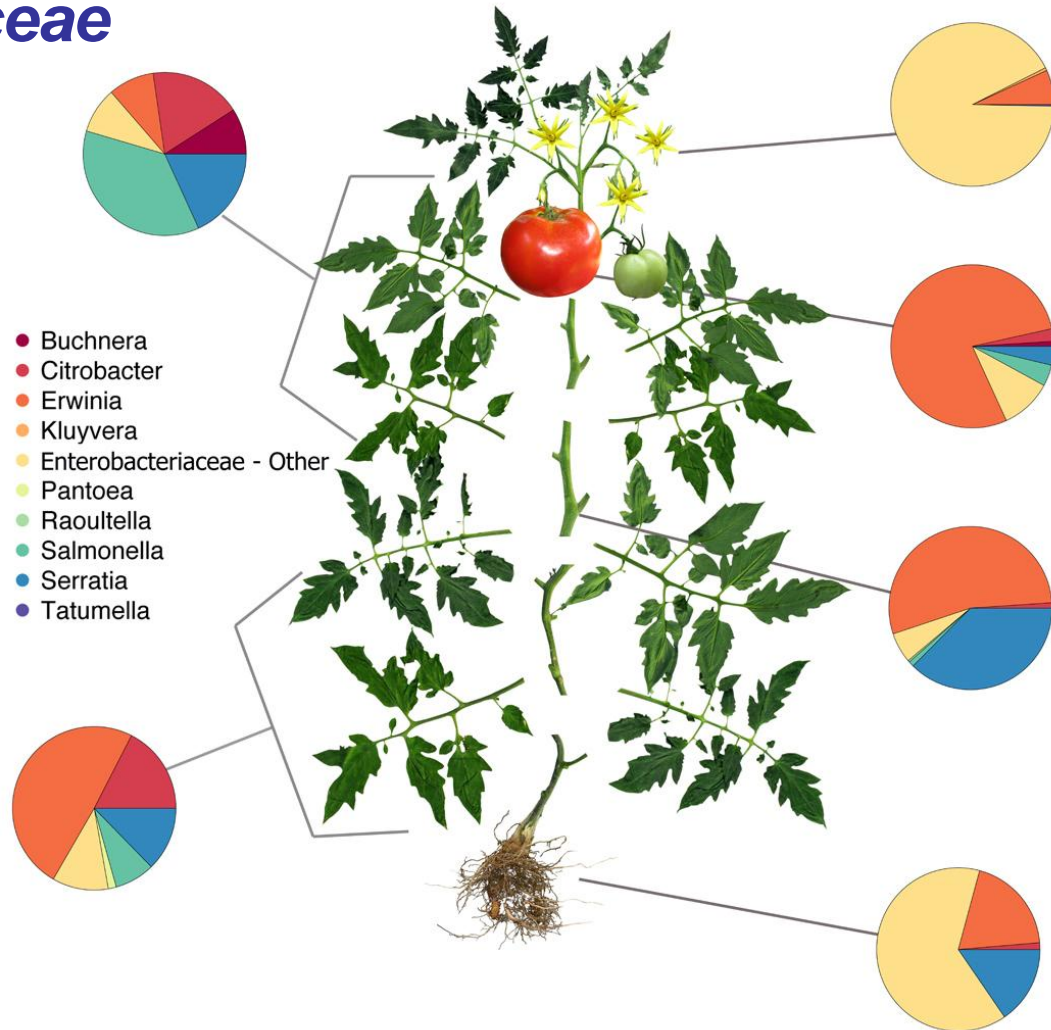


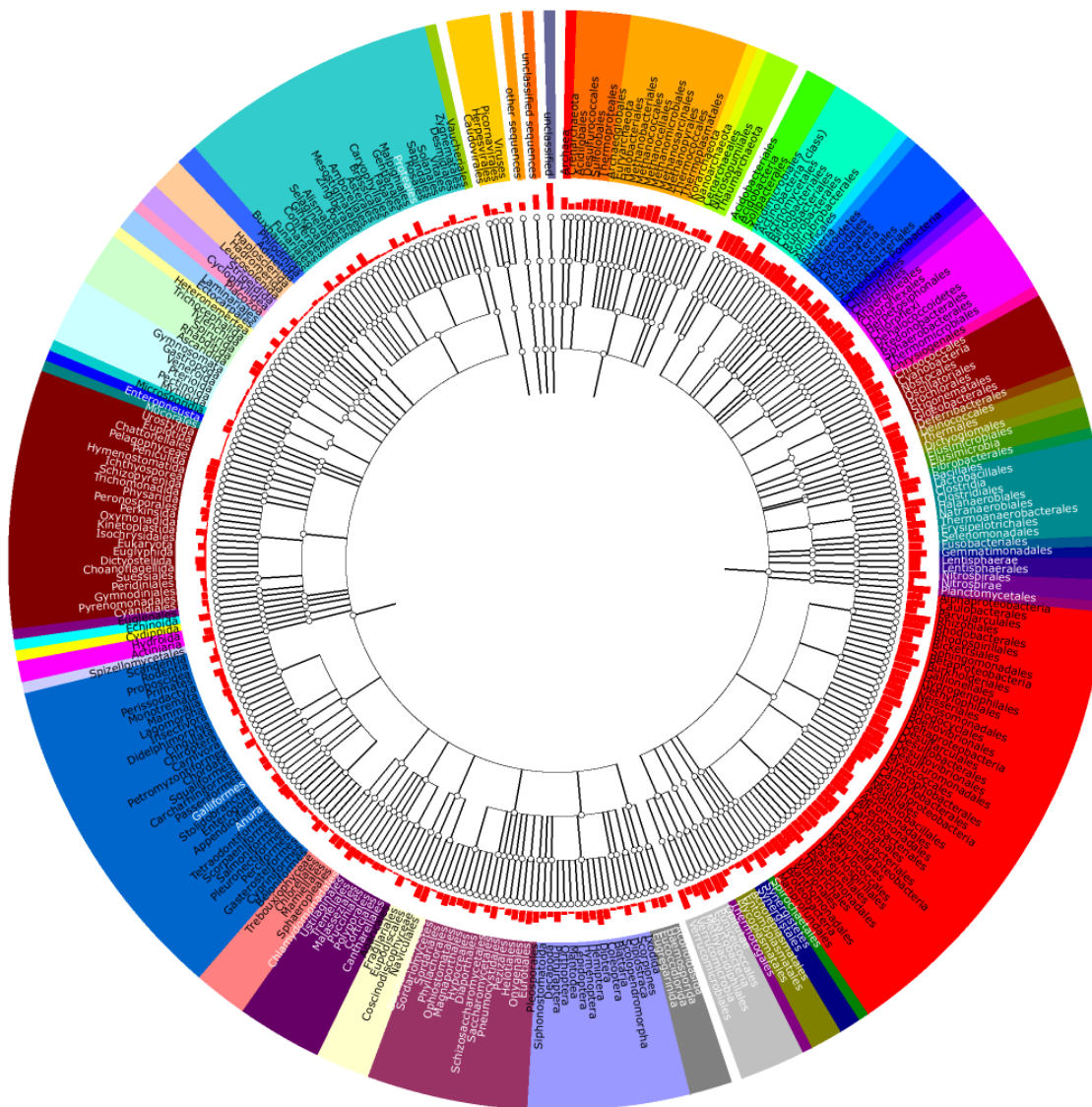
cultured



uncultured

Enterobacteriaceae

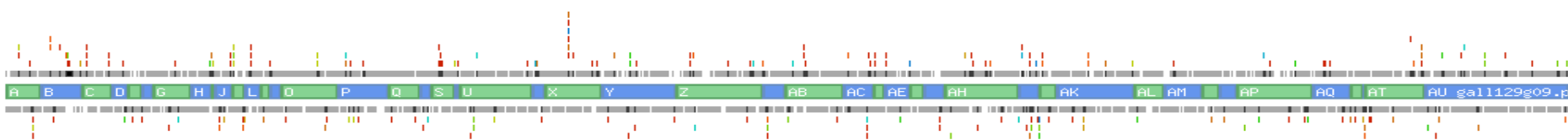




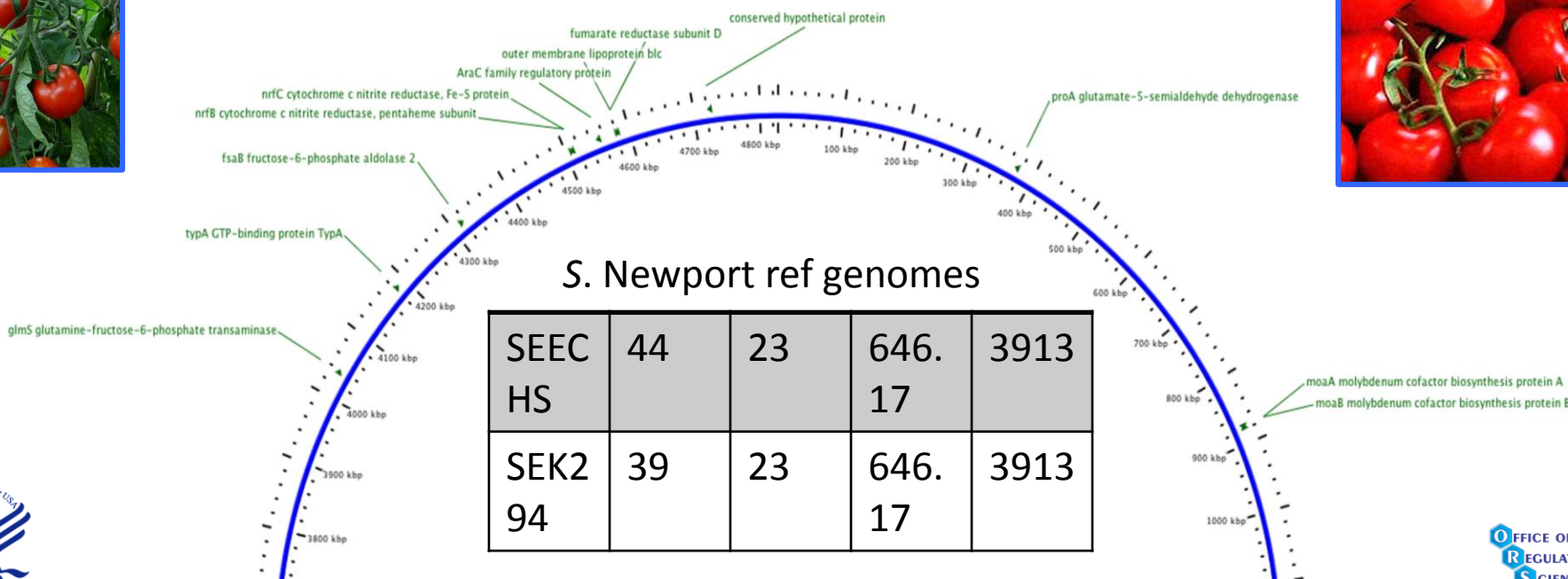
Phyla in a *Salmonella* Positive Metagenome

A Parallel Move Forward in Foods...


Metagenomic Identification of *Salmonella* in Tomatoes and Tomato Growing Environs





Salmonella hits from tomato phyllosphere metagenome





SUMMARY

- 

Various desktop NGS platforms now exist and are dropping precipitously in price – and per reaction cost making the technology largely accessible for public health applications
- 

Genomics is becoming an integral part of the science of food safety, both for more comprehensive characterization and testing of foods and for providing insight about the phylo-geographic structure of foodborne pathogens.
- 

WGS, as part of a laboratory next-generation analysis pipeline, can augment food safety investigations, particularly in cases where strain homogeneity is a problem
- 

Metagenomic analysis provides one avenue for the potential detailed typing of pathogens directly from complex high background food sources (*i.e.*, produce).
- 

Much can be gleaned from genomic and metagenomic approaches comparing outbreak and environmental strains, the microbial compositions of foods, and the rich meta-data associated with these important strains.

Thank you...



**ONE THING'S FOR CERTAIN:
IT TAKES A VILLAGE TO GET THERE! Many thanks to the following:**



Division of Microbiology-FDA



Marc Allard
Ruth Timme Narjol Gonzalez Yi Chen

Christine Keys *ORISE Fellows*

Rebecca Bell Andrea Ottesen

Charlie Wang Jie Zheng Sarah Allard

Division of Biostatistics-FDA James Pettengill

Errol Strain Yan Luo Cong Li

CDC

John Besser Eija Trees Patti Fields

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Mark Mammel Chris Elkins Scott Jackson

FDA Division of Field Sciences Rebecca Dreisch

NYPH Bill Wolfgang Kimberly Musser and colleagues

MPH Alvina Chu and colleagues

National Institutes of health

David Lipman (NCBI)

Tatiana Tatusova (NCBI)

William Klimke (NCBI)

VaTech



Steve Rideout

Office of Regulatory Science-FDA

USDA-ARS

Steve Musser

Pat Milner

