

“Regulatory Science, Food Safety, and the Integration of Genomic Sciences to Mitigate Current Pathogen Testing Challenges”



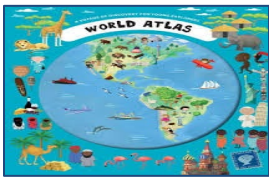
Presented by:
Eric W. Brown, Ph.D., M.Sc., FAAM
Director, Division of Microbiology

Center for Food Safety & Applied Nutrition
U.S. Food & Drug Administration
College Park, MD 20740 USA

Thursday September 5th, 2024

IFSH Annual Meeting – Rosemont, IL





The Complex and Global Etiology of Foods (& Their Pathogens)



Salad



Shrimp – India
Cilantro – Mexico
Romaine – Salinas, CA
Cheddar – Wisconsin
Carrots – Idaho
Gruyere – Switzerland
Pecans – Georgia
Sprouts – Chicago
Red Cabbage - NY

Sushi



Shrimp – Indonesia
Imitation Crab – Alaska
Tuna Scrape – India
Fish Roe – Seychelles
Salmon – Puget Sound
Soy Sauce – China
Rice – Thailand
Seaweed Wrap – CA
Avocado – Mexico
Cucumber – Maryland
Wasabi – Japan
Pepper – Vietnam

Fruit platter



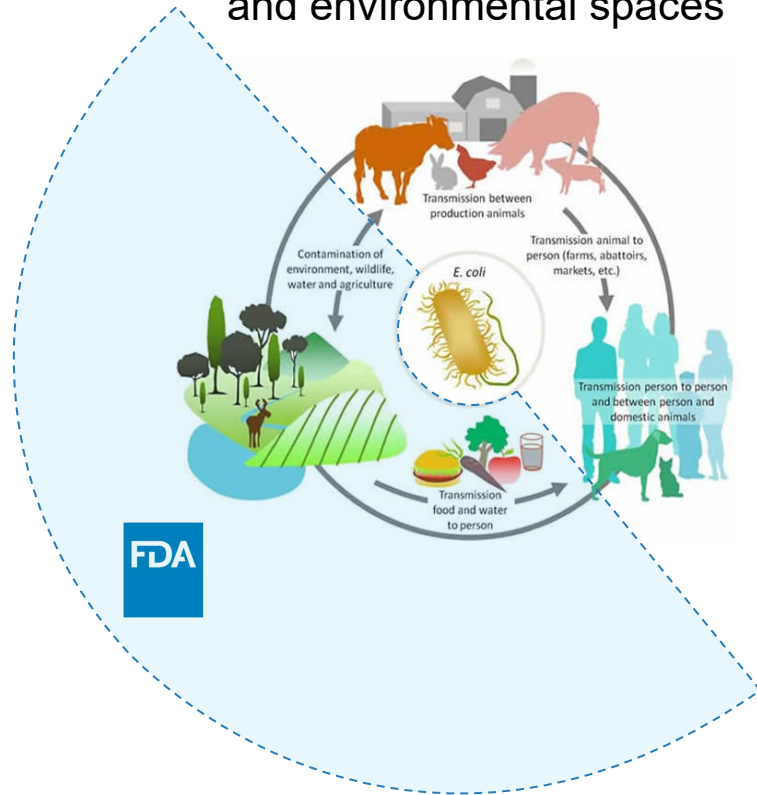
Watermelon – Delaware
Blackberries – Guatemala
Blueberries – New Jersey
Pineapple – Guam
Grapes – California
Kiwi – New Zealand
Apples – New York
Pears – Oregon
Cantaloupe – Costa Rica
Honeydew – Arizona
Papaya – Mexico
Banana – Costa Rica

GenomeTrakr: FDA's Genomic epidemiology program for enteric pathogens



One Health

CSFAN focus on surveillance of food and environmental spaces



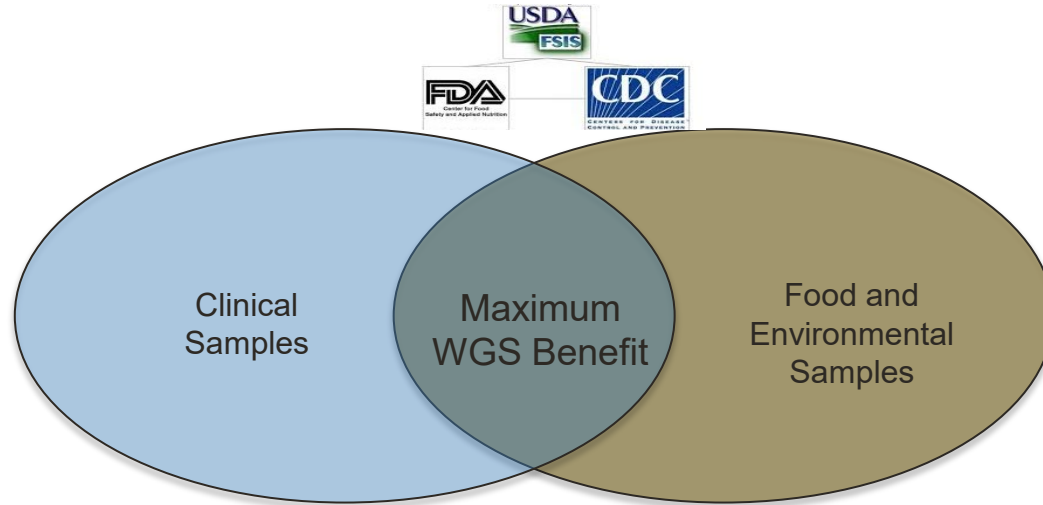
46 Directly Funded Labs:

- FDA labs
- LFFM WGS track funding (31 labs)
- Congressional Shrimp mandate funds
- Contract agreements



- CFSAN
- FDA field laboratory
- ★ GenomeTrakr Sponsored Laboratory

The Balanced (all-encompassing) Approach:



Genomic Signal

- PNUSAL000016 missing missing clinical
- FDA00008248 USA:IL 2014-08-14 mung bean sprouts
- PNUSAL001039 missing missing clinical
- FDA00008247 USA:IL 2014-08-13 sprout irrigation water
- PNUSAL000956 missing missing clinical
- PNUSAL000017 missing missing clinical
- PNUSAL000954 missing missing clinical
- PNUSAL000968 missing missing clinical
- PNUSAL000815 missing missing clinical
- PNUSAL000863 missing missing clinical

Epidemiological Signal



Traceback



Listeria enoki mushroom event cluster highlights global NGS contribution

103,465 Clusters currently tracked.



WGS: Direct Industry Application



Current Testing is Effective and Supportive: BUT Can we achieve additional value?

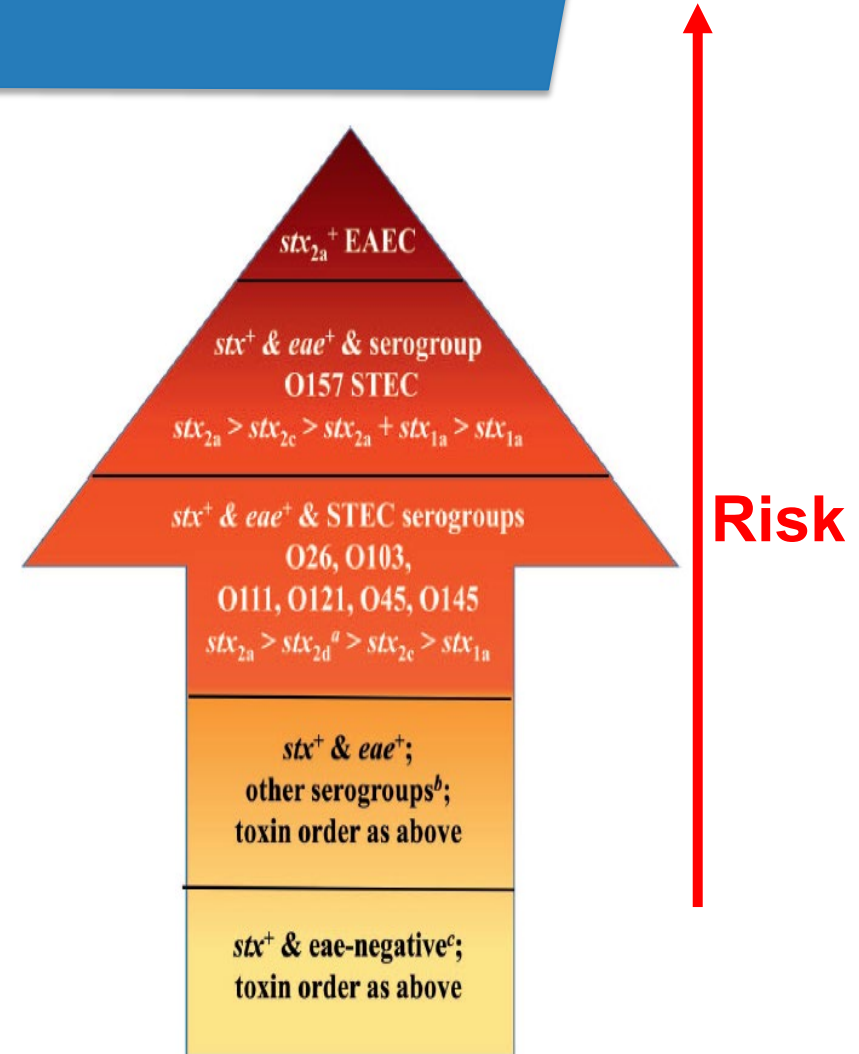
- That is, is there additional information that could drive a public health investigation, a risk assessment, or a compliance event further and/or faster by answering follow-up questions such as:
 - Is it possible to use genomic technology to predict a pathogen's risk and public health impact earlier and faster?
 - Is it possible to enhance identification of pathogen reservoirs and routes of transmission by applying environmental metagenomics as a complementary approach to longitudinal environmental microbiology studies?
 - Is it possible to employ genomic technologies to look deeper into microbiological sample enrichments to characterize strain diversity and specific population make-up of a pathogen (*ie*, not only to find *Salmonella* in an env sample but to find the *Salmonella* that caused a specific contamination event)?
 - It is possible to achieve precision food safety by using genomic tools to identify specifically acquired adaptations in persistent or troublesome strains so that preventive controls can be aimed precisely at a problem pathogen?



(1) Predicting a pathogen's risk and public health impact earlier and faster.



- > 400 STEC serotypes
- ~100 cause illness in humans
- Shiga toxin genes (*stx1* and *stx2*)
- Intimin (*eae*) and/or adherence gene (*aggR*)
- We need to determine *stx* and *eae* genes, serotype and virulotype →



Detection AND Identification

National Advisory Committee on Microbiology Criteria for Foods. J Food Prot. 2019;82(5):724-767.

Determining pathogenicity of *E. coli*



Third-generation sequencing (also known as long-read sequencing) is a class of DNA sequencing methods currently under active development....this sequencing works by reading the nucleotide sequences at the single molecule level, in contrast to existing methods that require breaking long strands of DNA into small segments then inferring nucleotide sequences by amplification and synthesis.



NANOPORE
MinION
High-throughput, high-sample number sequencing platform.

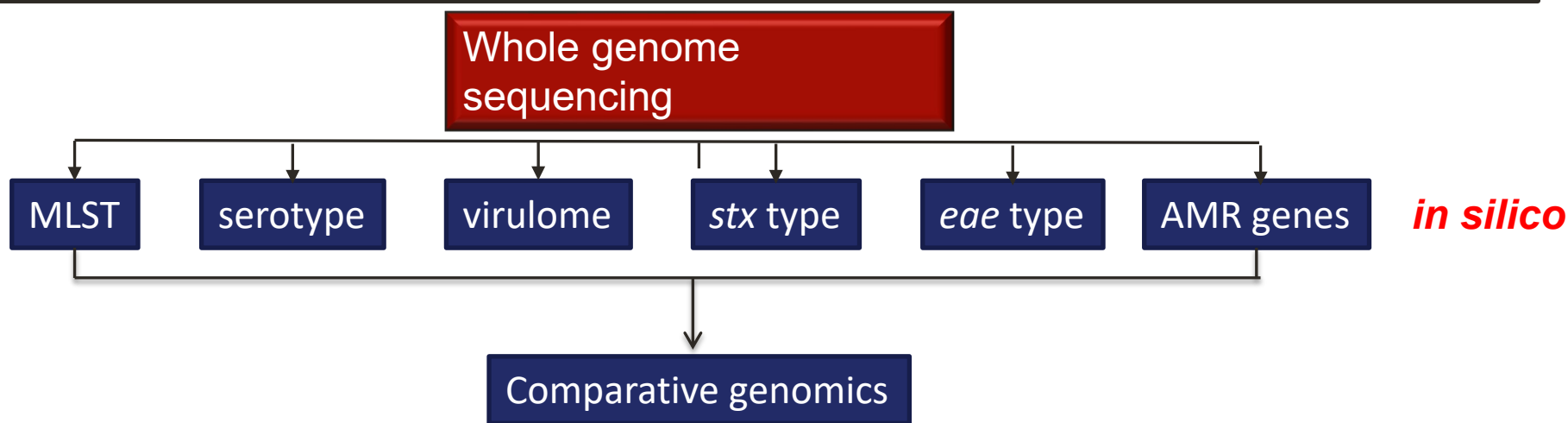


Long-read WGS provides virulence determination in unknown STECs in less than 4 hrs.



In silico characterization of the genomes of the STECs - serotype and virulence genes

strains	ST	serotype	stx1 type	stx2 type	eae type	espA	espB	espJ	espK	gad	astA	nleA	nleB	nleC	tir	pssA	air	tccP	cif	espF	espl	efa1	ehxA	espP	etpD	toxB	katP	subA	saa	sab
CFSAN046715	11	O157:H7	-	a	gamma-1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
FDA00009839	11	O157:H7	-	a	gamma-1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
CFSAN046724	21	O26:H11	a	-	beta-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	-	
IEH-NGS-ECO-00076	21	O26:H11	a	-	beta-1	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	
CFSAN046651	655	O121:H19	-	a	epsilon-2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	
FDA00010257	655	O121:H19	-	a	epsilon-2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	
CFSAN046652	677	Ounk:H21	-	d	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
CFSAN046748	677	O174:H21	a	d	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	+	
CFSAN046713	955	O139:H1	-	e	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
CFSAN051539	993	O100:H30	-	e	-	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
CFSAN051526	43	O6:H10	c	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	
CFSAN051527	43	O6:H10	c	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	



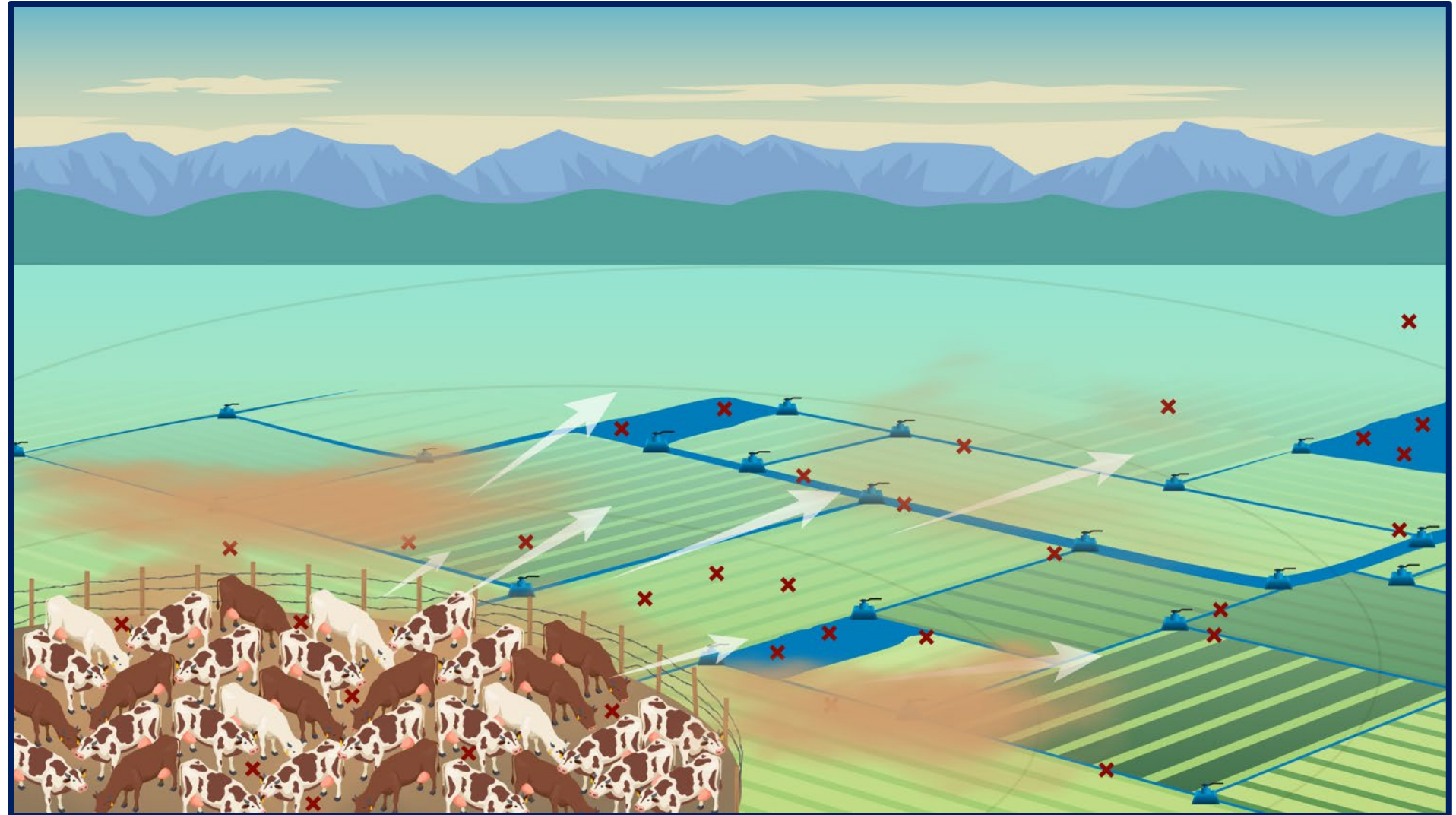
in silico

FDA stood up the **FSAC** Committee in 2019

Moving from Genes, to operons, to metabolic pathways, to Systems approaches

(2) Applying environmental metagenomics as a complementary approach to longitudinal environmental microbiology studies.

Pathogens and Adjacent/Nearby Land Use





Community of microbial organisms



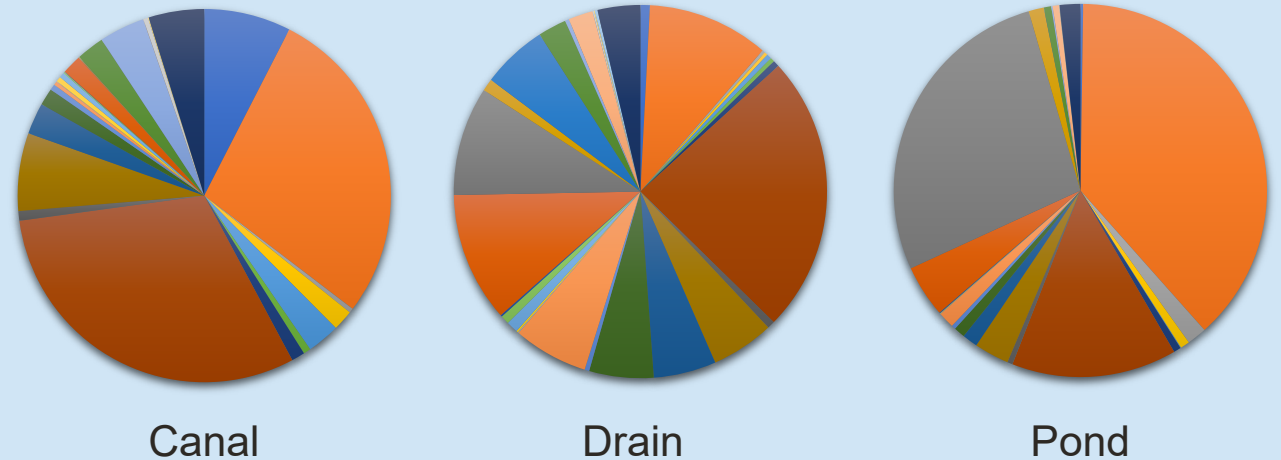
- Sequence DNA from the sample to identify all microorganisms in the sample microbial community and their abundances relative to each other (microbiome).
- Detect the presence of Shiga toxin genes and other *E. coli* virulence genes along with serogroups by performing metagenomic sequencing on samples culture enriched for *E. coli*.
- Identify host of scat samples using mitochondrial DNA sequence.

Applications of metagenomic sequencing in longitudinal studies

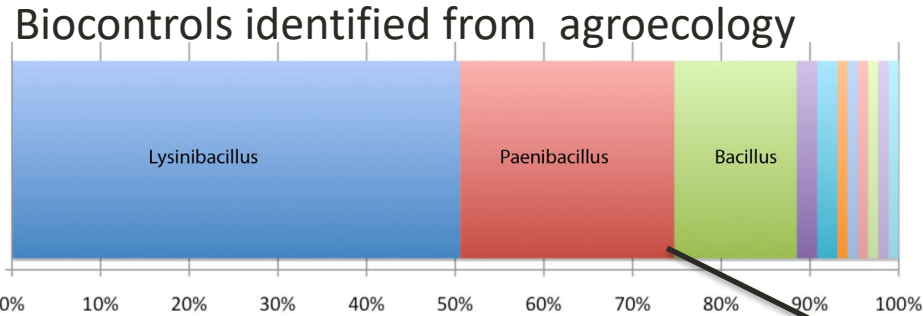
Metagenomics for Produce Safety

- Overall health of the various environmental sources and how that changes over time or with agricultural impacts
- How the localized geography, environmental factors, or activities impact water sources and whether this is correlated with pathogen presence or persistence.
- “Indicator” microbiomes that may be indicative of pathogen presence (co-associated) or may be inhibitory for pathogen growth.
- How sample collection methods influence information gained and best method for a particular application.

Enriched Water Microbiomes

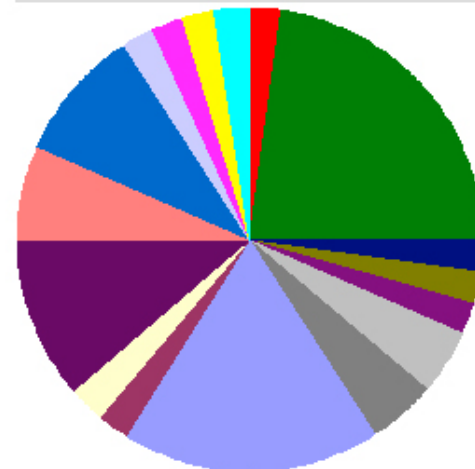


* Does CA enjoy a natural Biological Control state?



Enriched *Paenibacillus* spp.

Paenibacillus

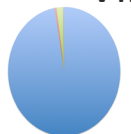


- Paenibacillus sp. 61724 2.27% (1)
- Paenibacillus taiwanensis 22.73% (10)
- Paenibacillus turicensis 2.27% (1)
- Paenibacillus ginsengisoli 2.27% (1)
- Paenibacillus sp. H10-05 2.27% (1)
- uncultured Paenibacillus sp. 4.55% (2)
- Paenibacillus sp. HS16-12 4.55% (2)
- Paenibacillus panaciterrae 18.18% (8)
- Paenibacillus sp. MCRI 90 2.27% (1)
- Paenibacillus sp. HSCC 1657 2.27% (1)
- Paenibacillus sp. F4 11.36% (5)
- Paenibacillus sp. JA-08 6.82% (3)
- Paenibacillus sp. JS15-08 9.09% (4)
- Paenibacillus sp. GT08-03 2.27% (1)
- Paenibacillus anaericanus 2.27% (1)
- Paenibacillus sp. B519 2.27% (1)
- Paenibacillus odorifer 2.27% (1)

California

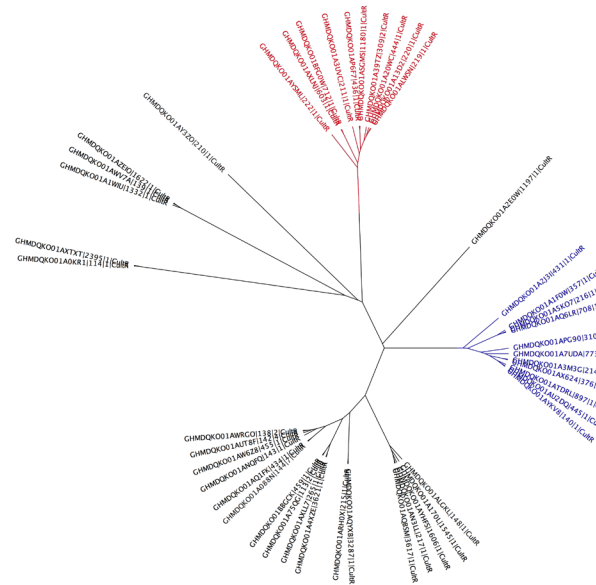


Virginia

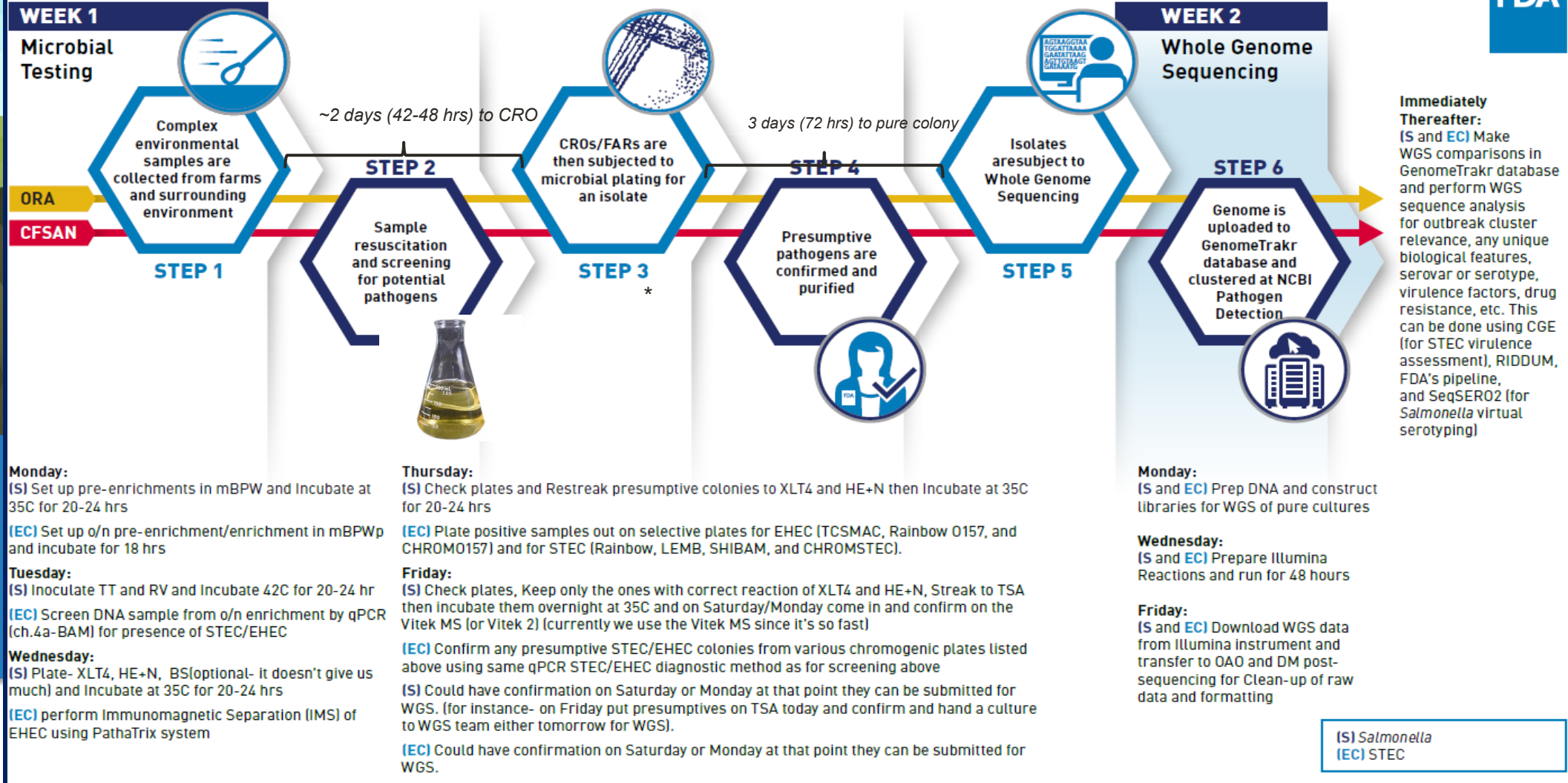


Florida

- PROTEOBACTERIA
- ACTINOBACTERIA
- FUNGI/METAZOA
- FIRMICUTES



Bacterial Pathogen Workflow

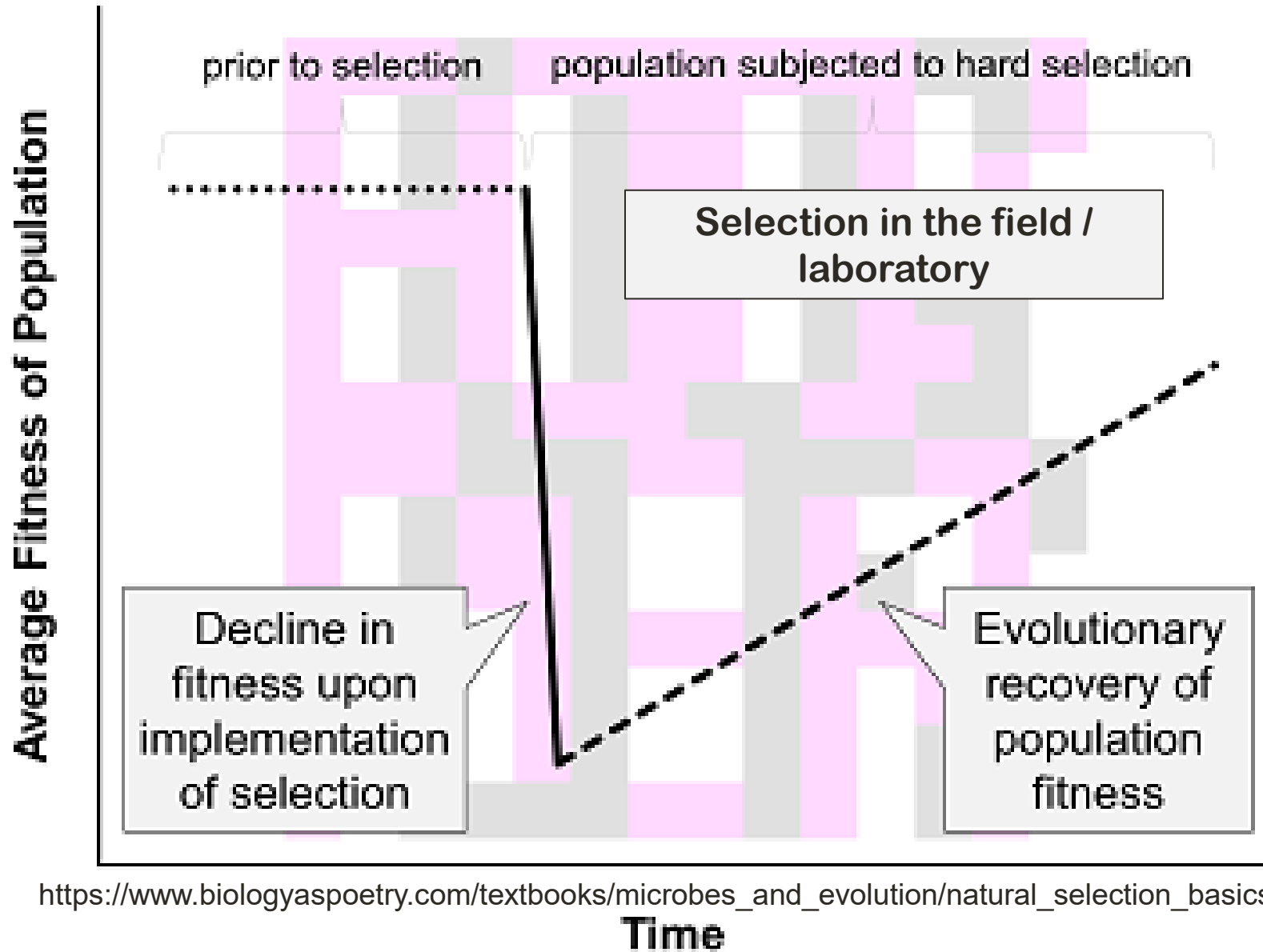


FDA
(STEC/EHEC/SALMO)

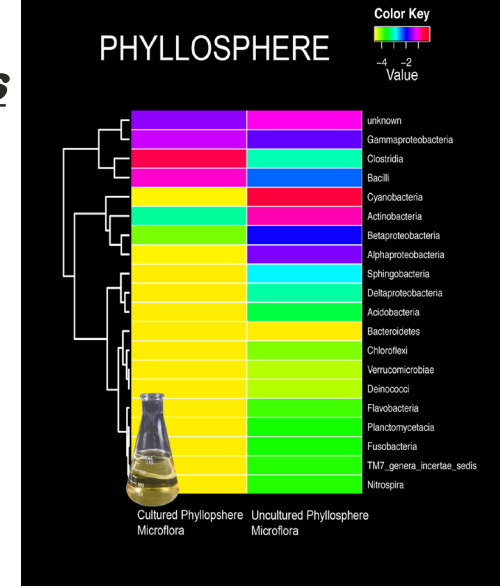
(3) Looking deeper into microbiological sample enrichments to characterize strain diversity and specific population make-up of a pathogen.



Different Strain Fitness Is Likely Linked to Changing Environmental Conditions



https://www.biologyaspoetry.com/textbooks/microbes_and_evolution/natural_selection_basics.html



Salmonella Outbreak Linked to Onions

 Food Safety Alert

Posted October 20, 2021

Fast Facts

- Illnesses: 652 (60 new)
- Hospitalizations: 129 (13 new)
- Deaths: 0
- States: 37 (1 new)
- Recall: No
- Investigation status: Active



CAUTION: SALMONELLA NEWPORT OUTBREAK CAUSED BY ONIONS

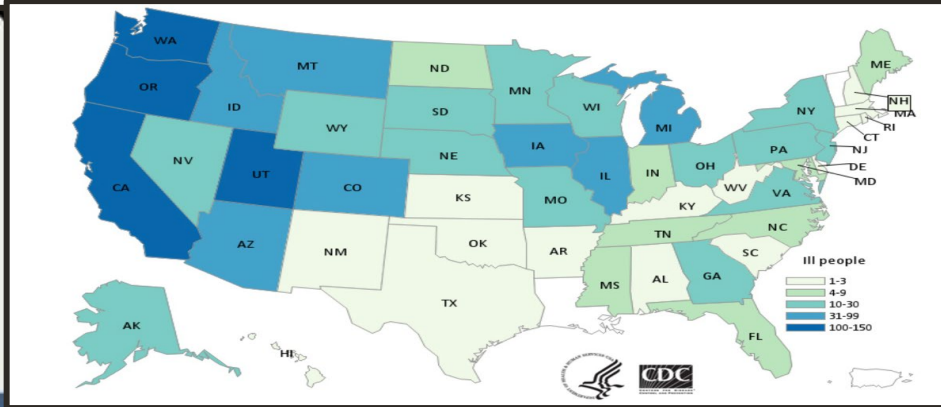
red, yellow, white and sweet yellow onions connected to Salmonella Newport outbreak.

As a health precaution, please throw away your onions if you are not sure of their source.



OUTBREAK UPDATE SALMONELLA INFECTIONS

WHAT TO DO Read the updated public health notice and follow the advice in it to help reduce your risk of a Salmonella infection.



CANADA.CA/HEALTH

Canada

ONION SALMONELLA OUTBREAK

- 640 CASES IN 43 STATES
- CASES IN CT, NJ & NY
- 85 PEOPLE HOSPITALIZED NATIONWIDE
- NO DEATHS REPORTED



RECALLS RED, WHITE, YELLOW & SWEET YELLOW ONIONS



87 5:36 PM



Food Safety News

FDA reports on Salmonella outbreak linked to red onions

FSN



WHAT ONIONS ARE IMPACTED

- Fresh, whole red, white, and yellow onions sold in grocery stores throughout the U.S.
- Imported from Chihuahua, Mexico
- CDC investigating other onions and suppliers

ONIONS BEHIND NATIONWIDE SALMONELLA CASES



10:08

77

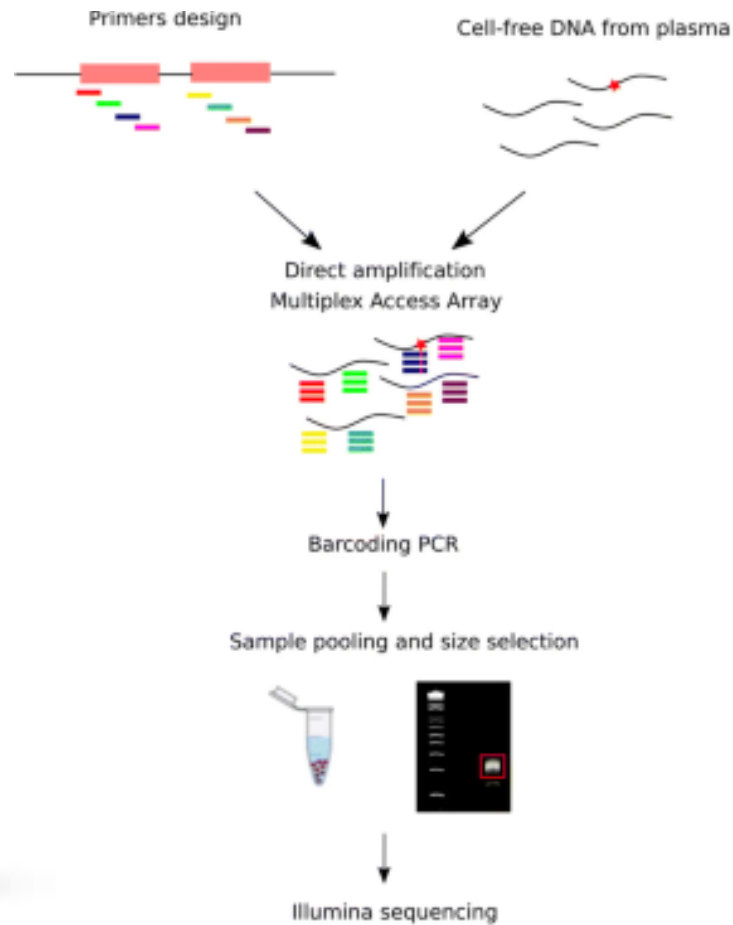
TOP US NEWS:

targeted amplicon sequencing: how does it work?

Targeted amplicon sequencing is a next-generation sequencing (NGS) method that uses polymerase chain reaction (PCR) to amplify specific DNA fragments, or amplicons, from a genome.

The process involves the following steps:

1. Design primers
2. Transform samples into libraries
3. Amplify DNA
4. Multiplex samples
5. Sequence amplicons



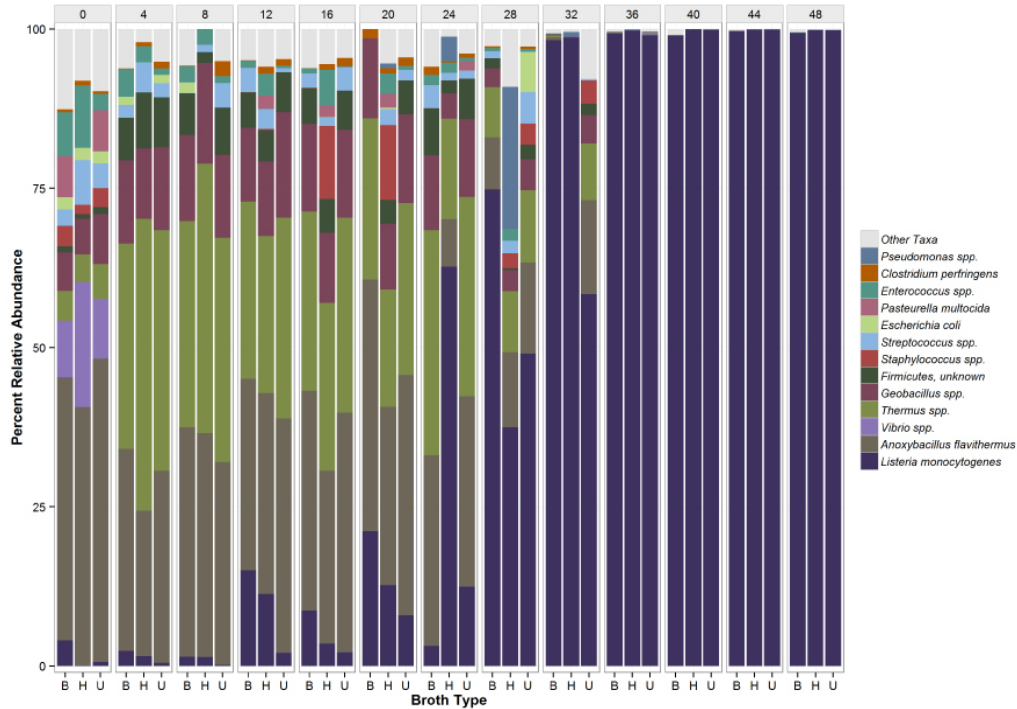
Targeted amplicon sequencing can identify individual strains in a population and characterize them down to a molecular epidemiologically relevant level.

Shotgun Metagenomics and Quasi-Metagenomics:

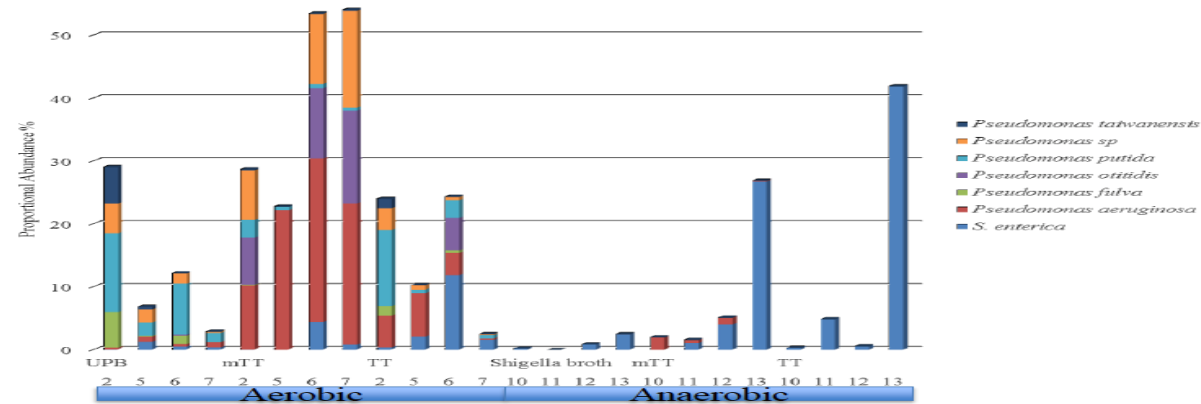
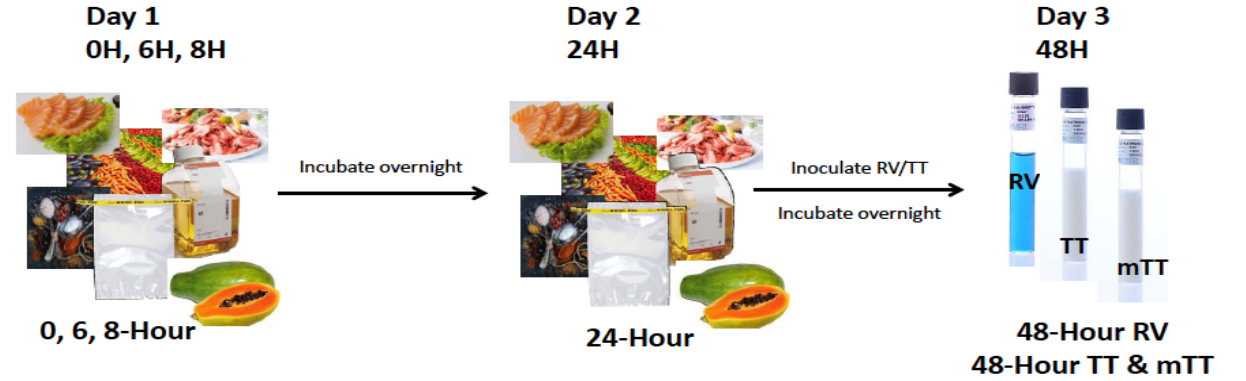


TOWARDS A CULTURE INDEPENDENT FOOD TESTING PROGRAM

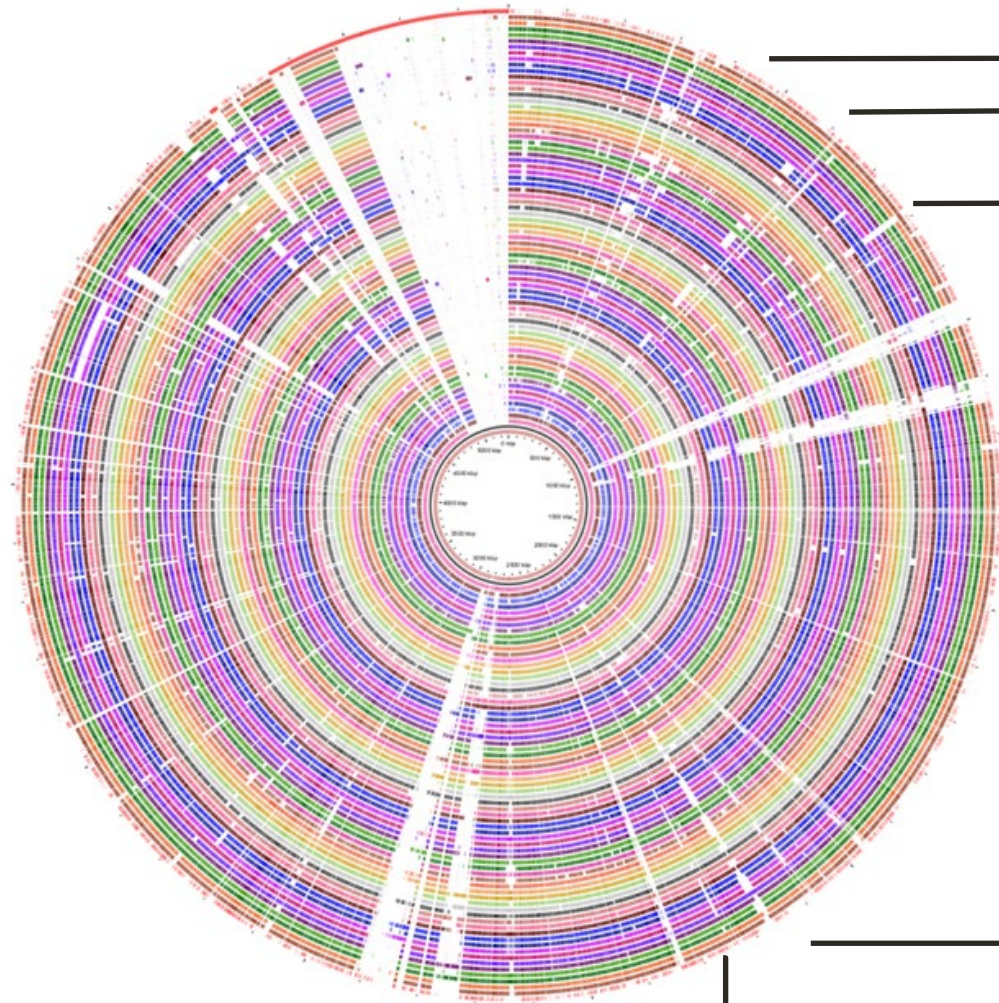
Lm enrichment (BAM) – characterized every 4 hours to describe genomic coverage of *Lm* and describe co-enriching microbiota



METAGENOMICS



Salmonella Baits Panel (SBP) Design (v0.07)



Pan genome
LT2- Reference
Salmonella Heidelberg

Custom *Salmonella* baits
(Twist Bioscience)

Total of 13,330 baits. ~10K
Salmonella specific baits

~3000 serovar specific baits

Baits cover ~ 34% of the genome
which are diagnostic markers for
the top 30 serovars.

Baits- *Salmonella* specific

Baits- LT2 serovar specific

(4) Achieving precision food safety by using genomic tools to identify specifically acquired adaptations in persistent or troublesome strains .



- **Environmental Monitoring and Root Cause Analysis for Farms and RCR Crop Production Ecosystems**
- **Genome-enhanced Risk Assessment**
- **Effectiveness of cleaning and sanitization/Enhanced Preventive Controls/Precision Food Safety**
- **Mapping if Adaptive Changes in *Salmonella* and other pathogens**
- **Cracking the “persistence code”**

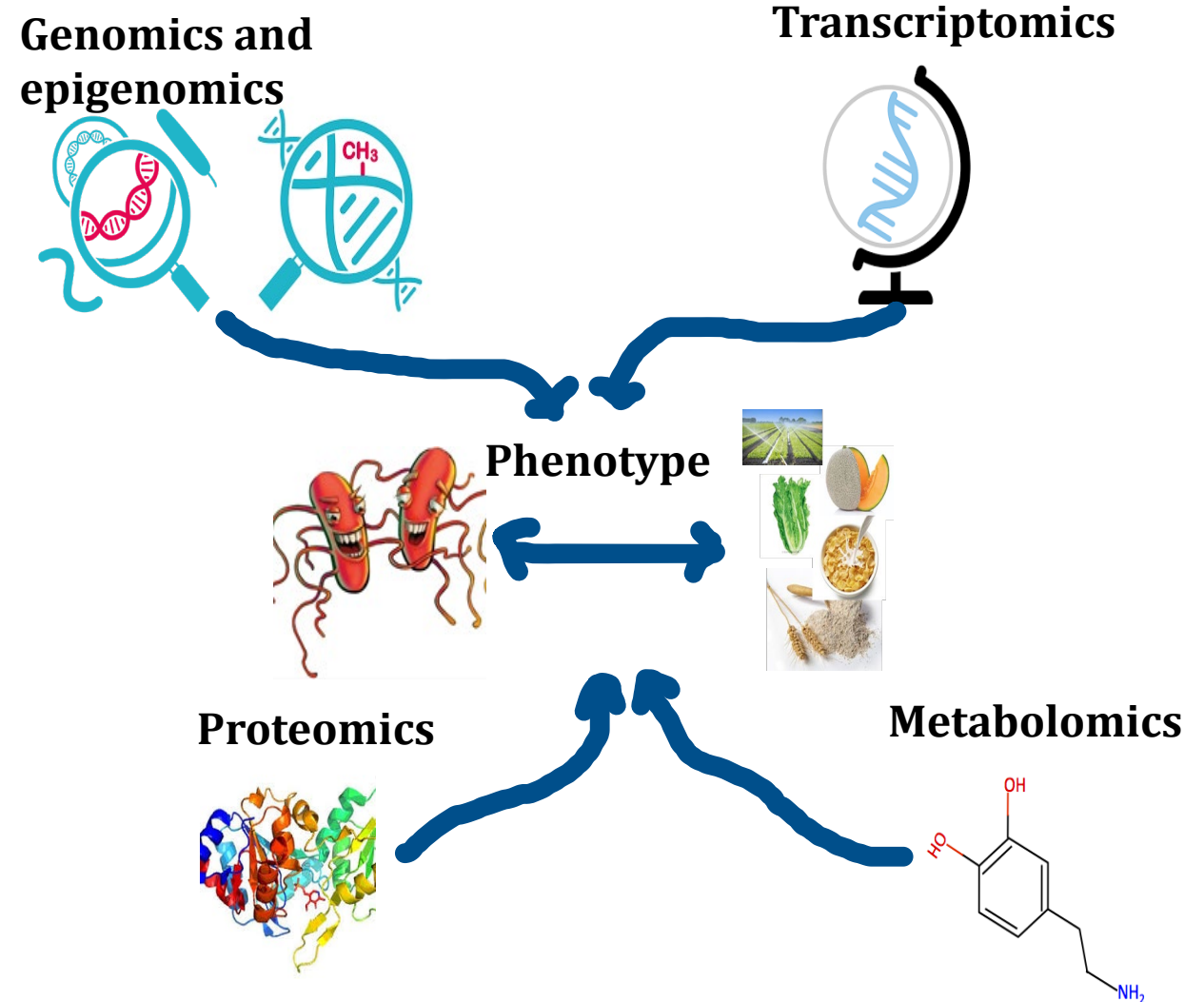
**Prescriptive
Precision
Mitigation**

Genomics and the Rise of Precision Food Safety



Functional Genomics

- ④ The study of how genes and intergenic regions of the genome contribute to different biological processes or environments
- ④ Represent links between genotype (genome wide) and phenotype, or metagenome and community phenotype



Food Safety Questions

Phenotypes

- Persistence
- Heavy Metal Resistance
- Disinfectant Resistance
- Acid Resistance
- Thermal Tolerance
- Desiccation Tolerance
- Biofilm Persistence



Bioprocess or environment

Production



Processing

Distribution

Retail



Research Paper

Salmonella Survival Kinetics on Pecans, Hazelnuts, and Pine Nuts at Various Water Activities and Temperatures

SOFIA M. SANTILLANA FARAKOS

¹U.S. Food and Drug Administration, Bedford Park, Illinois

MS 16-392: Received

Survival of Salmonella during Production of Partially Sprouted Pumpkin, Sunflower, and Chia Seeds Dried for Direct Consumption

Research Paper

SUSANNE E. KELLER,^{1*} NATHAN M. ANDERSON,¹ CAN WANG,² STEPHEN T. RURRICK,^{1,†} IAN M. HILDEBRANDT,^{1,†} LAUREN J. GONSALVES

¹U.S. Food and Drug Administration, 6502 South Archer Road, Bedford Park, Illinois

MS 17-318: Received



A Quantitative Assessment of the Risk of Human Salmonellosis Arising from the Consumption of Almonds in the United States: The Impact of Preventive Treatment Levels

SOFIA M. SANTILLANA FARAKOS,^{1*†} RÉGIS POUILLOT,^{1†} RHOMA JOHNSON,¹ JUDITH SPUNGEN,¹ INSOOK SON,¹

Research Paper

Fate of Salmonella throughout Production and Refrigerated Storage of Tahini

YANGJUNNA ZHANG,¹ SUSANNE E. KELLER,² AND ELIZABETH M. GRASSO-KELLEY^{1*}

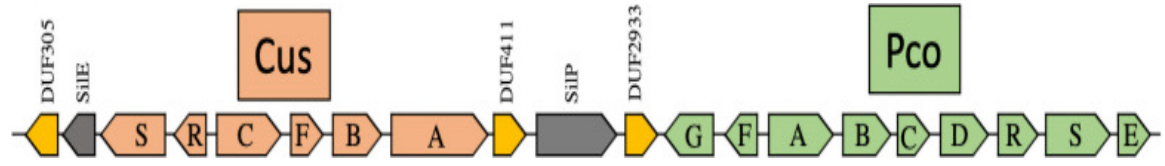
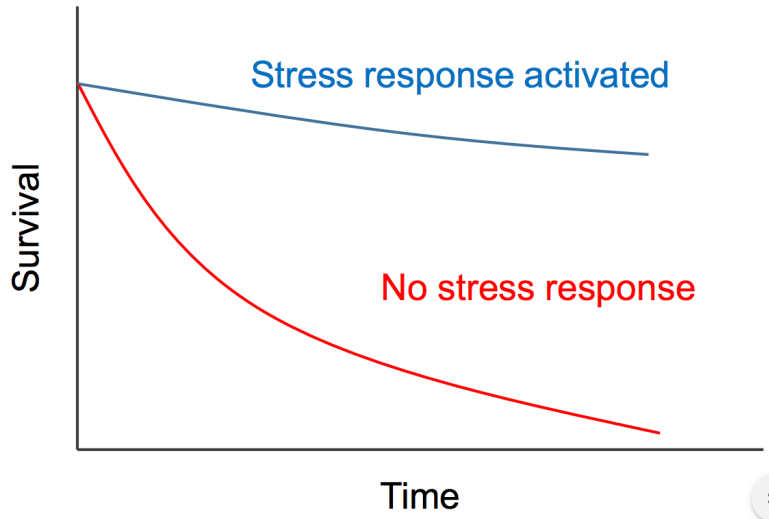
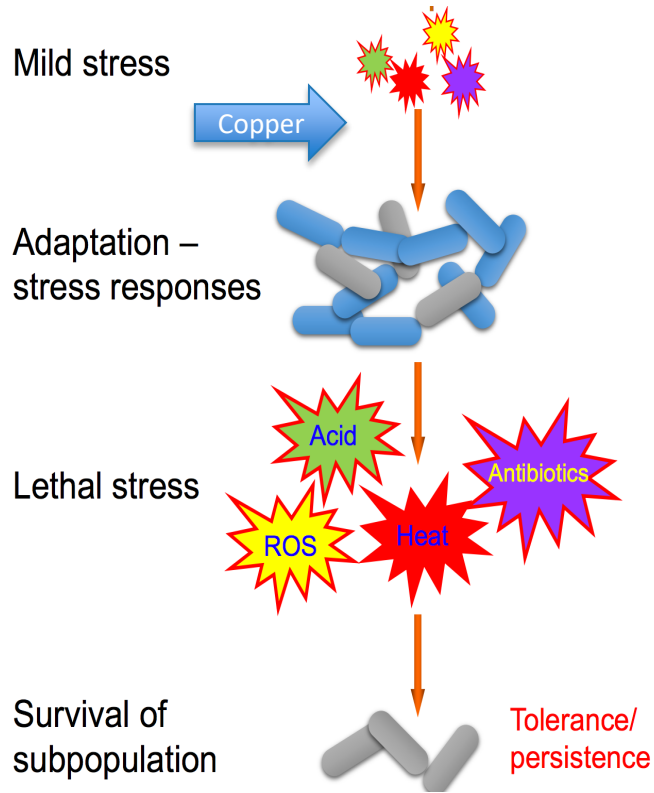
¹Illinois Institute of Technology, Institute for Food Safety and Health, 6502 South Archer Road, Bedford Park, Illinois 60501; and ²U.S. Food and Drug Administration, 6502 South Archer Road, Bedford Park, Illinois 60501, USA



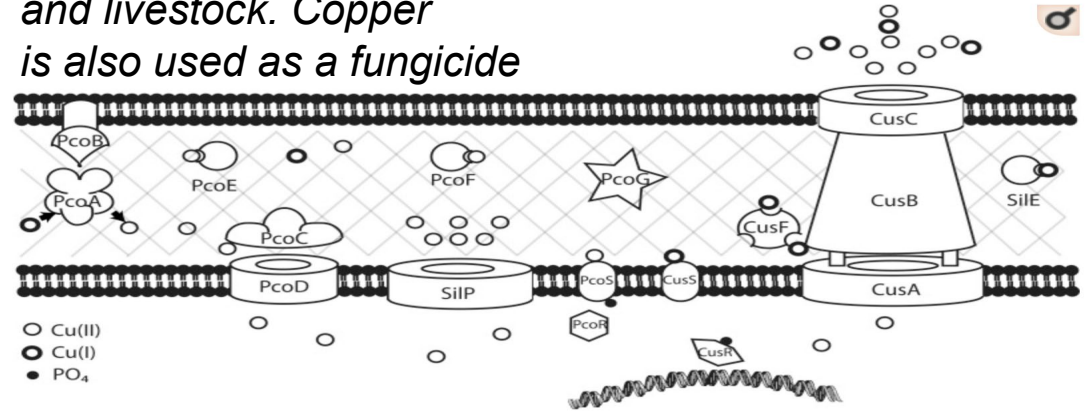
¹U.S. Food and Drug Administration



CHASRI Copper and Silver Resistance Islands: Stress Responses of *Salmonella* Montevideo and *S. Senftenberg*



In farming, copper soil treatments and feed are used to correct copper-deficient soils and livestock. Copper is also used as a fungicide



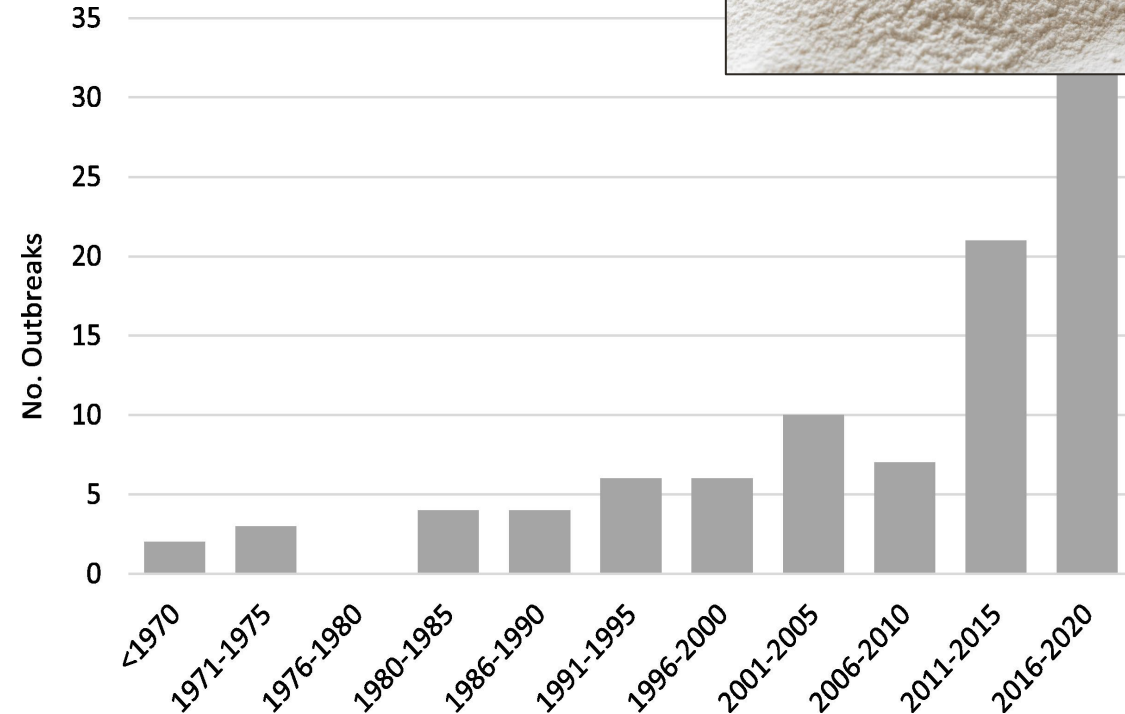
Pathogen Adaptation in LMF



Increasing occurrences of outbreaks associated with low moisture food (LMFs)

2012 - 2020

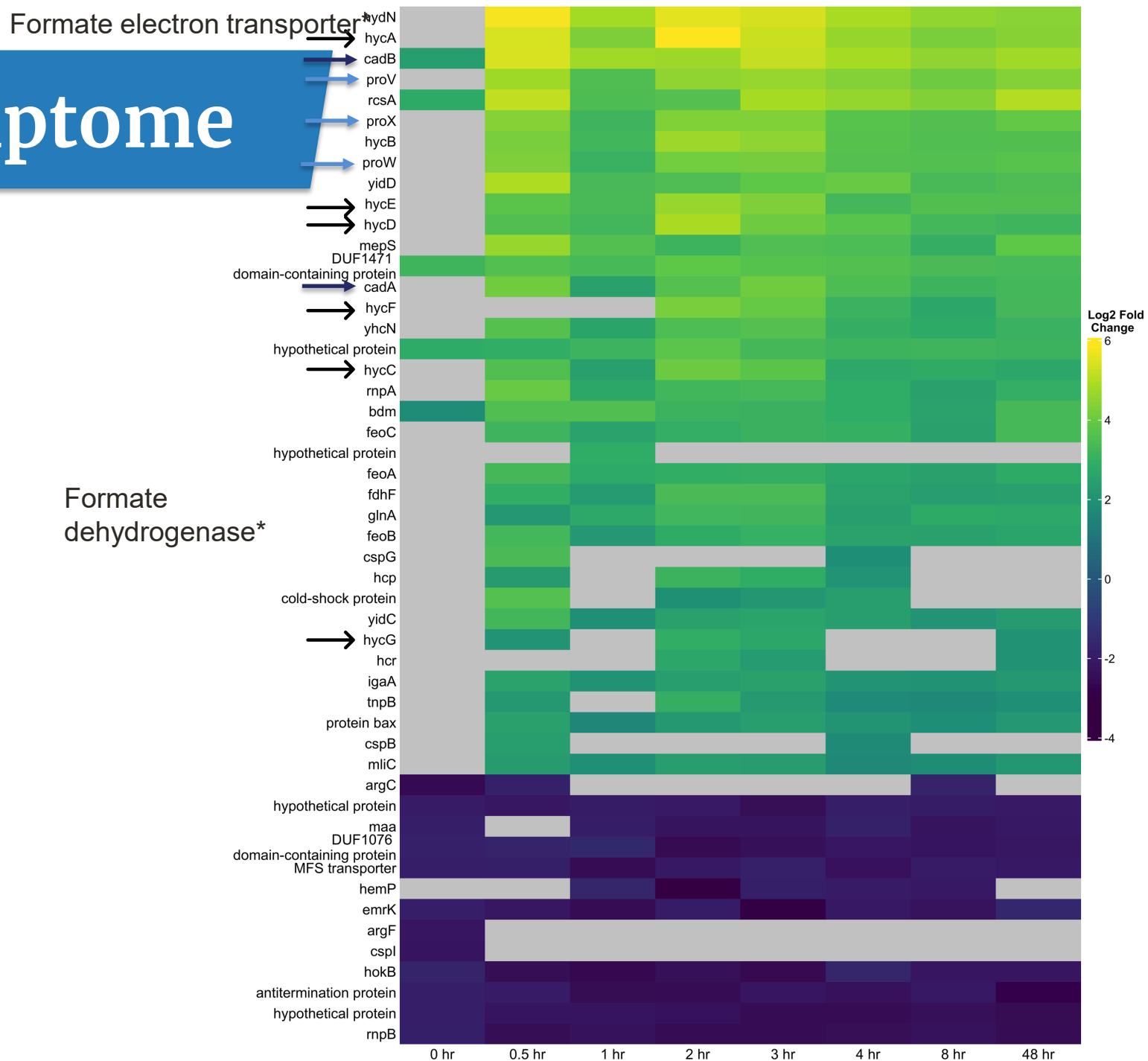
- 54 outbreaks
 - 44 – *Salmonella*
 - 7 – *E. coli*
- 1659 recalls
 - 1393 – *Salmonella*
 - 74 – *E. coli*



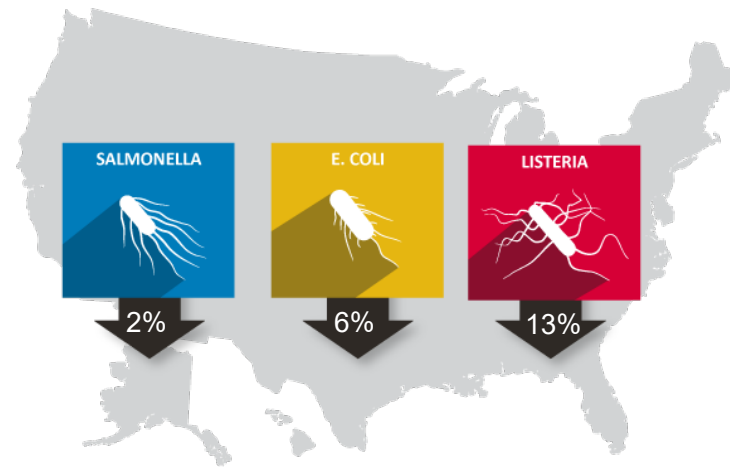
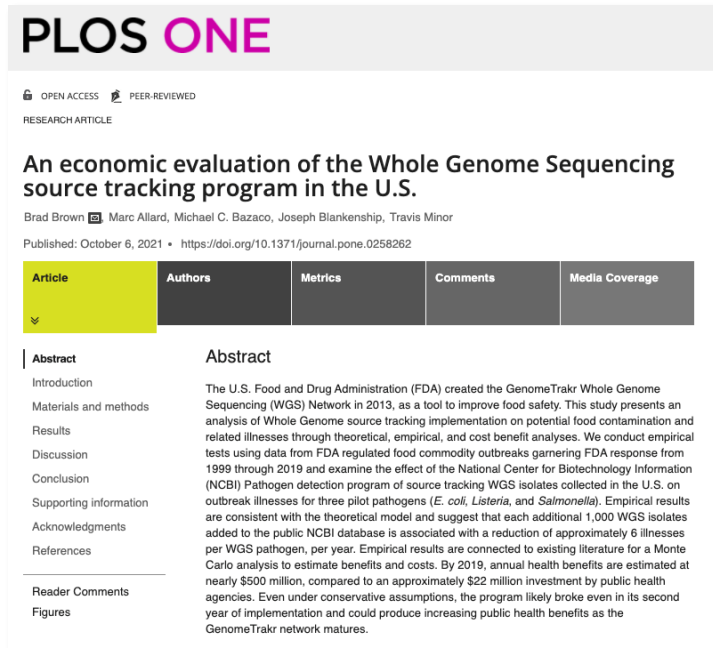
Distribution of outbreaks associated with low-moisture foods (LMFs)

E. coli O121 Transcriptome

- All results represent significantly DEG, $|L_2FC| > 1$
 - ($p_{adj} < 0.05$)
- *hyc* operon
 - Formate hydrogenlyase system
 - Anaerobic metabolism
- *cad* operon
 - Low pH/O₂ stress response
 - Reduces acid toxicity
- *pro* operon
 - Osmolarity stress response
 - Helps retain intracellular water
- Highly upregulated genes maintain high expression levels throughout

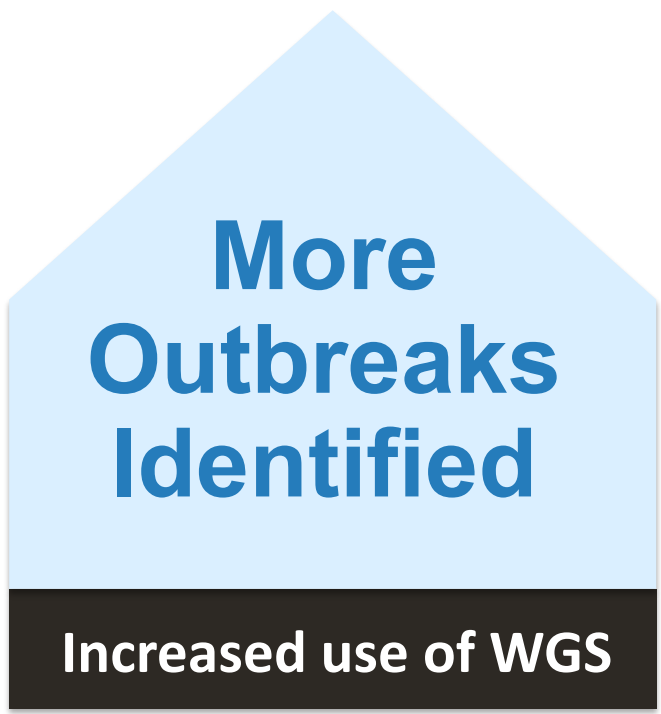


- GenomeTrakr program was likely cost effective by its second year of implementation
- \$100 M -> \$450 M in net annual health benefits (est. from 2019). Currently >\$1B annual savings.



- **Return on Investment:** \$10 dollars in averted human health costs for every \$1 dollar invested
Brown et al. (2021) An economic evaluation of the Whole Genome Sequencing source tracking program in the U.S. PLoS ONE 16(10): e0258262.

WGS Surveillance Outcome



How much additional illness can be averted with the 4 major advancements discussed here today?



Division of Microbiology (circa 1964 – 2024)

FDA



Thank you!