



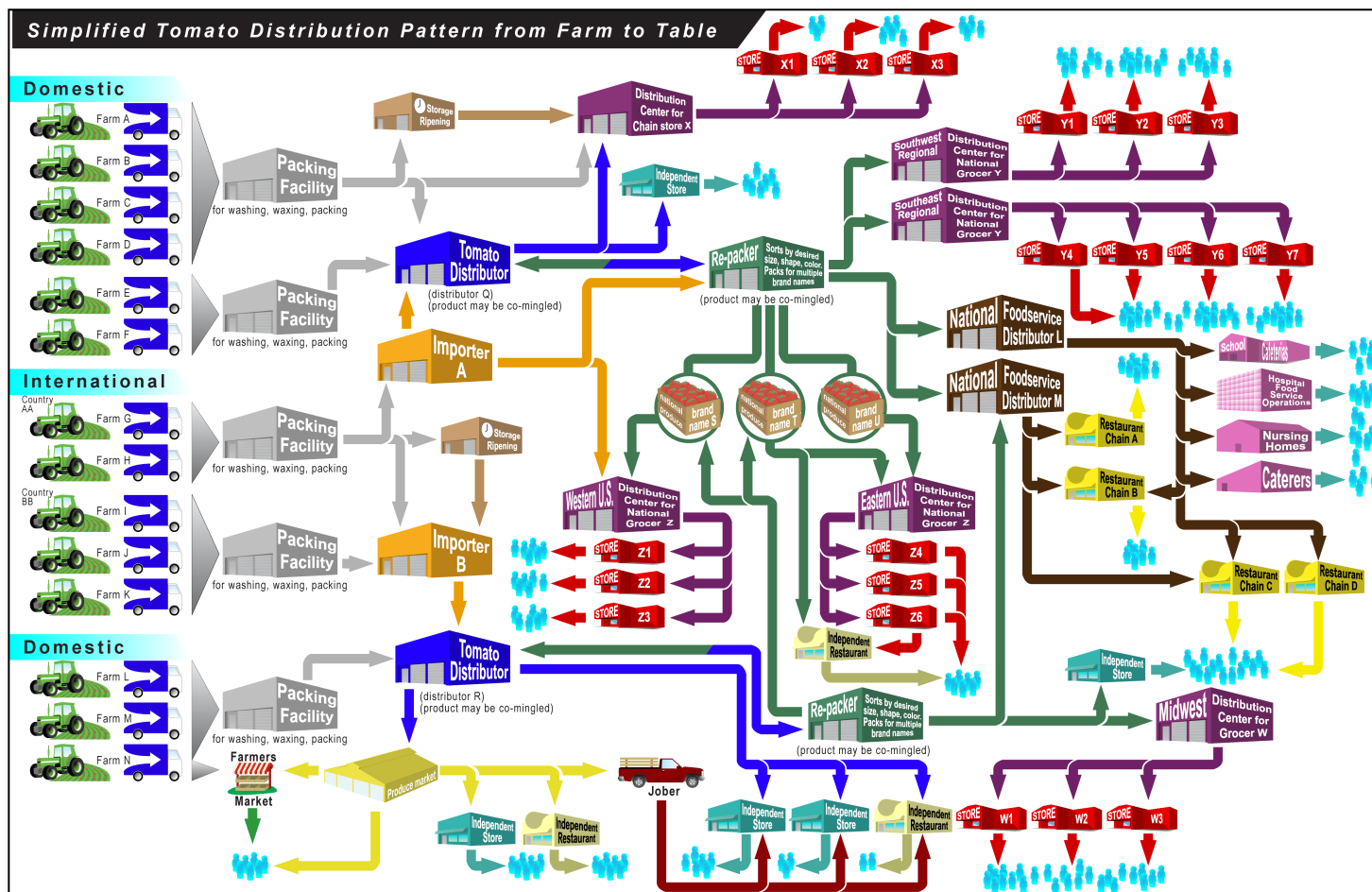
# How Whole Genome Sequencing is Augmenting FDA's Role in Foodborne Outbreak Investigation

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# *Safe, Wholesome, Sanitary Foods*



# The fresh-cut tomato supply chain





# COMPLEX FOOD VEHICLES



## LETTUCE

Canada, Chile, Dominican Republic, Mexico, Peru, USA



## CUCUMBERS

Canada, Honduras, India, Mexico, Spain, USA



## FETA CHEESE

Canada, Denmark, Egypt, Germany, Greece, Israel, Italy, Turkey, UK, USA



## VINAIGRETTE

Argentina, Brazil, Canada, Chile, China, France, Germany, Greece, India, Indonesia, Italy, Mexico, Morocco, Peru, Portugal, Spain, Thailand, Tunisia, Turkey, USA, Vietnam



## OLIVES

Greece, Israel, Mexico, Spain, USA



## SPROUTS

Argentina, Australia, Bangladesh, Canada, China, Egypt, France, India, Morocco, Nepal, Pakistan, South Africa, Spain, Turkey, USA



## MANDARIN ORANGES

Israel, Mexico, Morocco, South Africa, Spain



## CROUTONS

Argentina, Australia, Brazil, Canada, China, France, India, Mexico, Netherlands, Poland, Russia, Switzerland, Uruguay, USA, Vietnam



## TOMATOES

Canada, Dominican Republic, Holland, Israel, Italy, Mexico, USA



## ONIONS

Canada, China, Germany, India, USA



## The Well-Traveled Salad.

### Do You Know Where Your Food Has Been?

As consumers, many of us fail to recognize that even our domestic and local food supplies are part of a global network. The daily activity of consuming food directly links our health as humans to the health of crops and produce, food animals, and the environments in which they are produced.

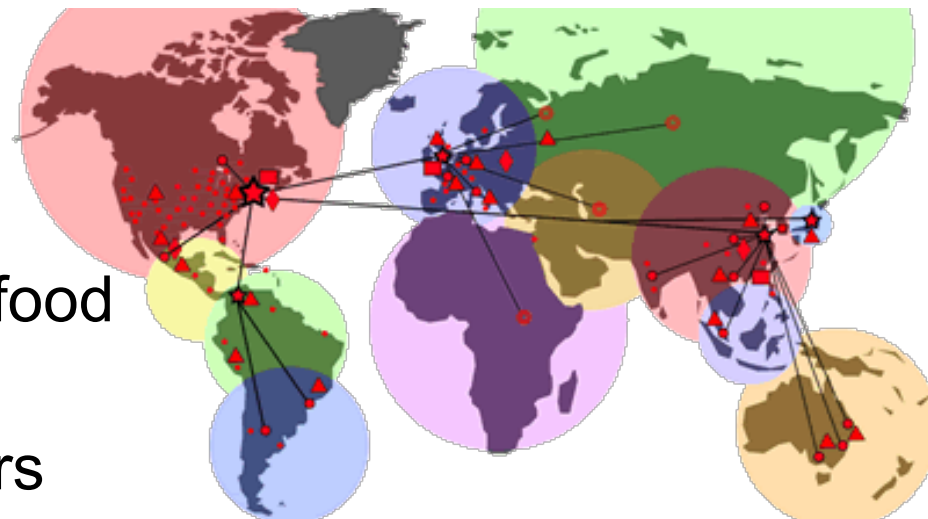


A "One Health" approach to food safety—bringing together expertise and resources from the clinical, veterinary, wildlife health, and ecology communities—has the potential to reveal the sources, pathways, and factors driving the outbreaks of foodborne illness and possibly prevent them from occurring in the first place.

NOTE: Countries are listed in alphabetical order and not by volume of export.

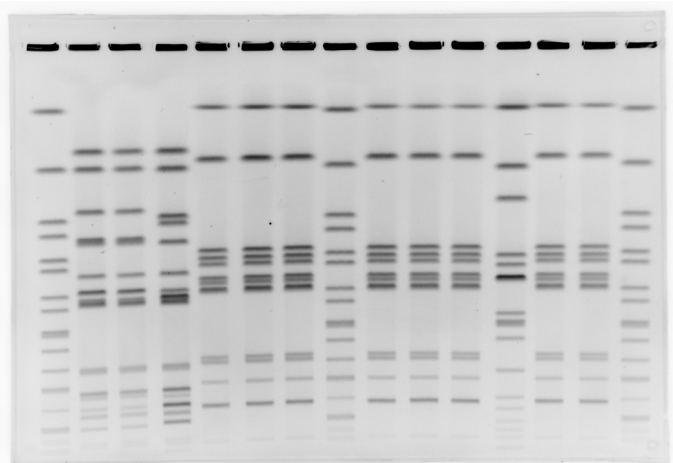
# Why develop a WGS-based Network?

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



# PFGE discrimination level

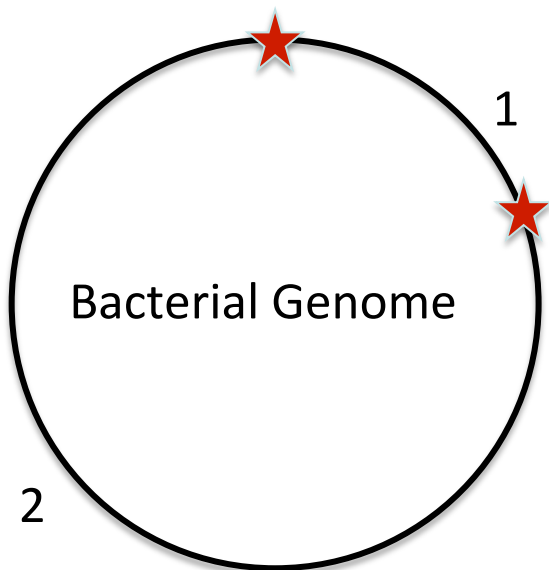
- Molecular technique that uses restriction enzymes to cut DNA at specific locations
- The resulting pieces are then run on a gel and separate according to size (with the smaller fragments moving further).
- Use the banding pattern of the gel to group bacterial isolates



# WGS:

## Whole-genome sequencing

- PFGE only gives us information at a restriction site based on the banding pattern
- WGS has the ability to give us information at every position in the bacterial genome

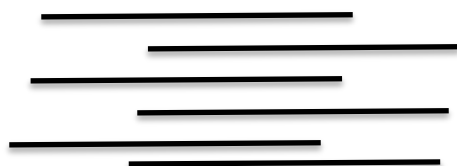


# WGS: Very brief overview

Bacterial  
Genome



Amplify genomic DNA and prepare for sequencing chemistry

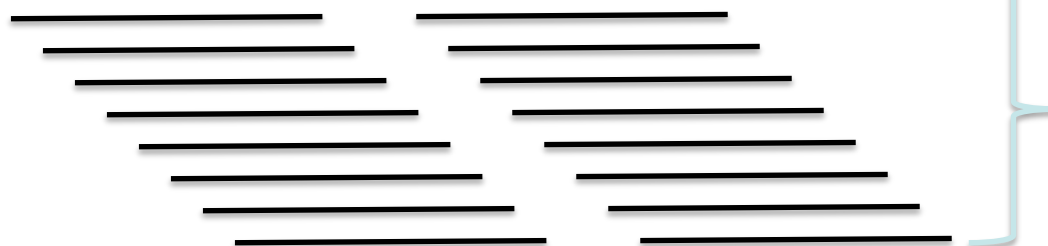


“Reads”

(thousands to millions)  
(range from 300 bp to 40 kb)



Assembly Method (many to choose from)



Aligned reads

Derived Genome Sequence



# WGS:

## Not all of the genomic sequence is needed

In fact, we expect many of the sites to be the same

SNP = Single Nucleotide Polymorphism

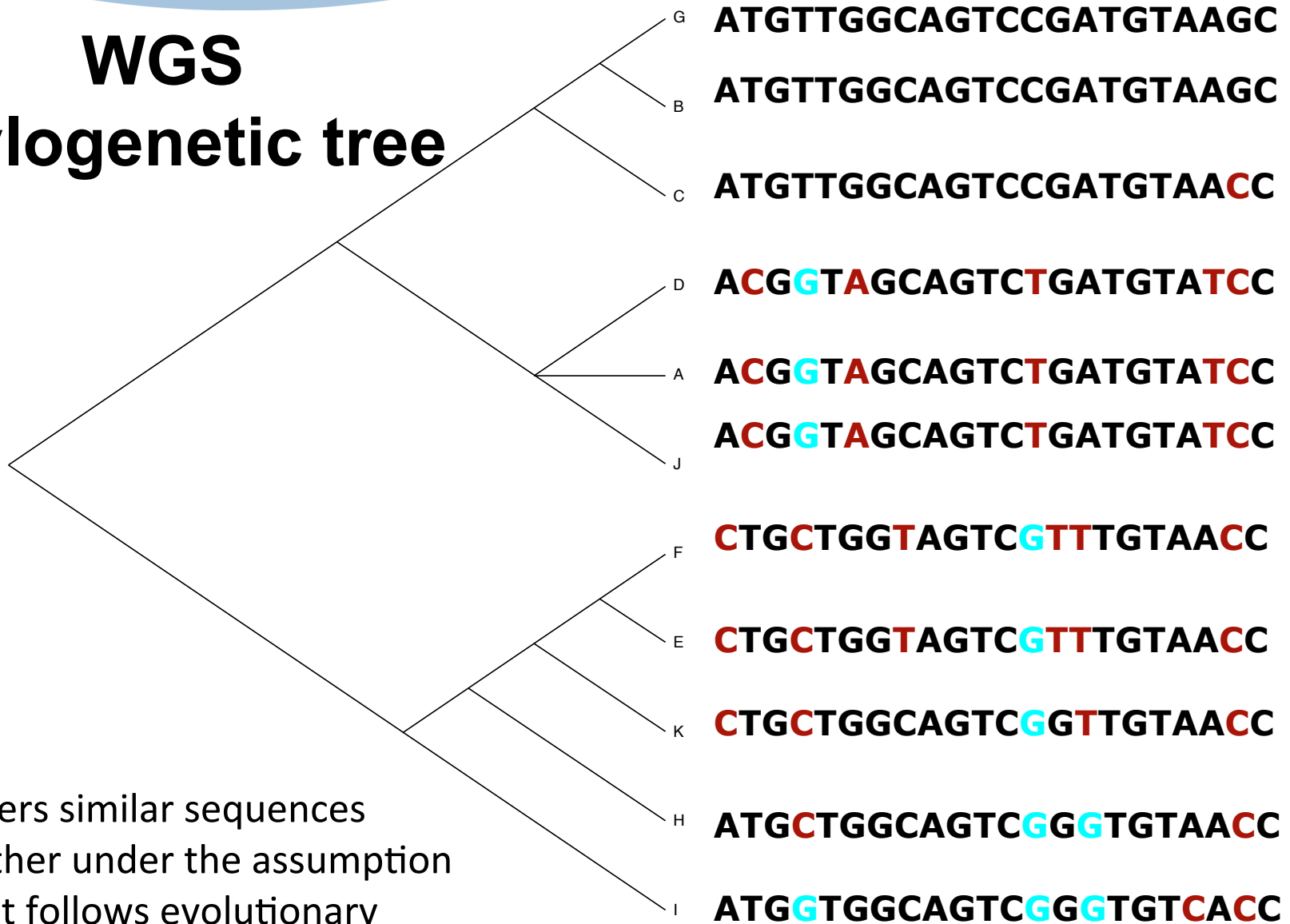


**T**TGTTGGCAG  
ATGT**A**GGCAG  
ATGTTGGCAG  
ATGTTGGCA**C**



4 aligned genome sequences  
reduced to 3 SNPs

# WGS Phylogenetic tree



Clusters similar sequences together under the assumption that it follows evolutionary history

# FDA's WGS program

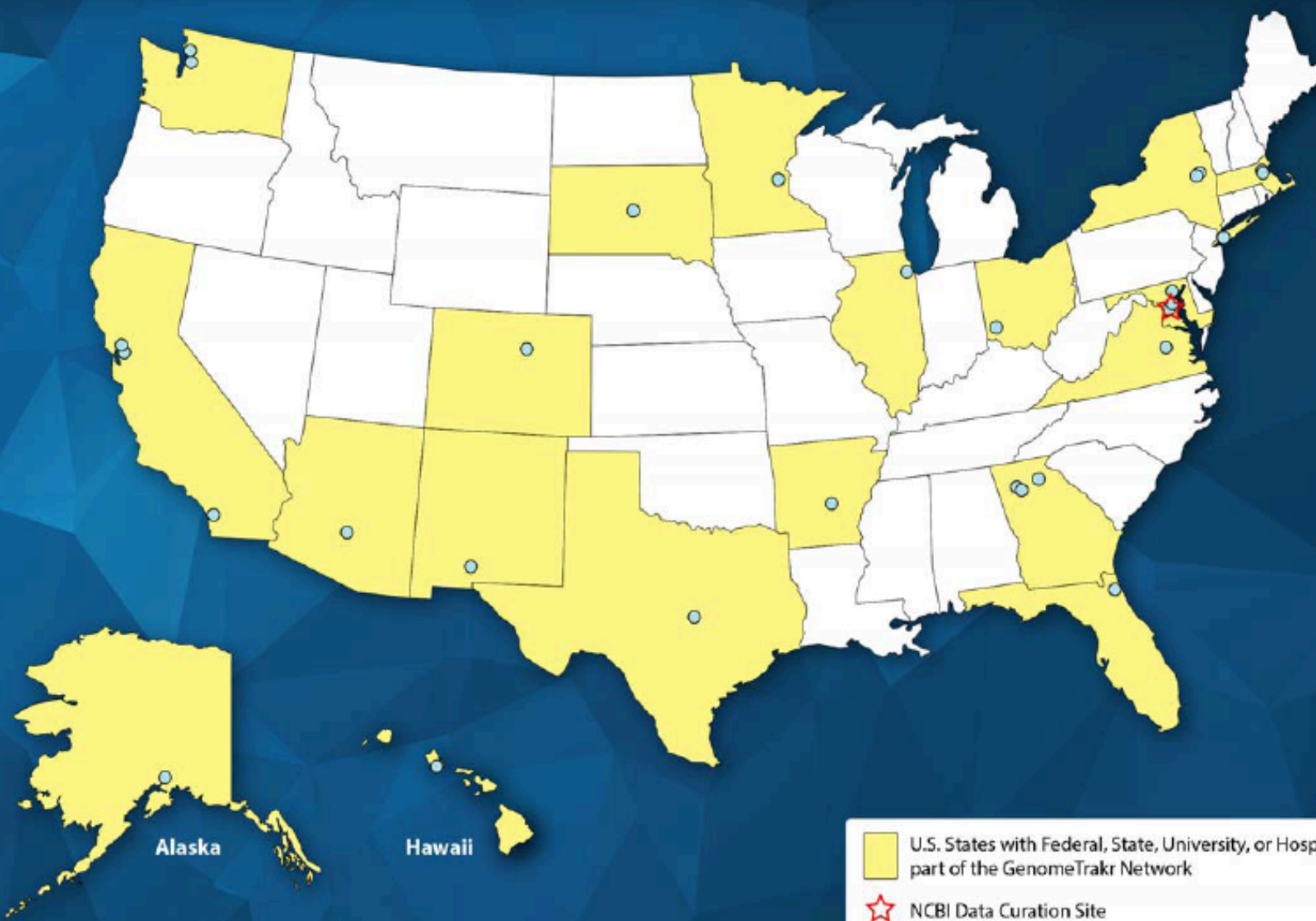
- Most basic application is identifying pathogens from food or environmental samples and then comparing it to clinical isolates
- WGS allows better resolution than PFGE in differentiating related versus unrelated strains
  - **Key for epidemiological insights**
- However, one of the most promising public health benefits may come from the combination of a pathogen's genomic information with its geographic location

# FDA's GenomeTrakr

- First distributed network of labs to use whole genome sequencing
- Consists:
  - 14 federal labs
  - 15 state health and university labs
  - 1 U.S. hospital lab
  - 9 international labs
  - Independent researchers
- Data curation and bioinformatic support/analyses provided by National Center for Biotechnology Information (NCBI)





# U.S. GenomeTrakr Labs



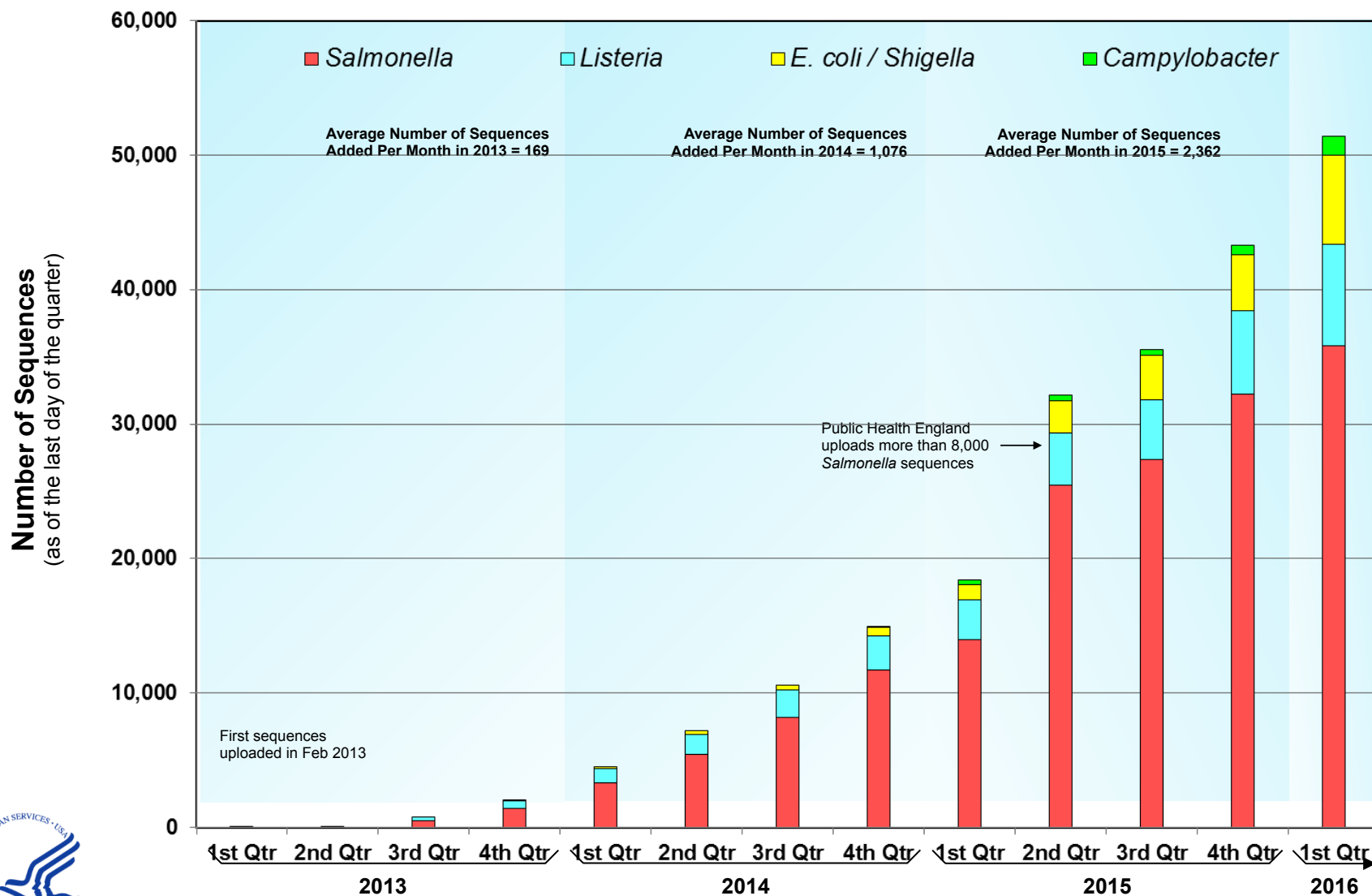
# Labs Outside the U.S. Contributing to GenomeTrakr



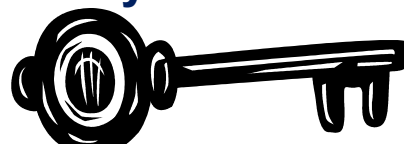
 Countries other than the U.S. with labs contributing to the GenomeTrakr Network

 Lab Locations

## Total Number of Sequences in the GenomeTrakr Database

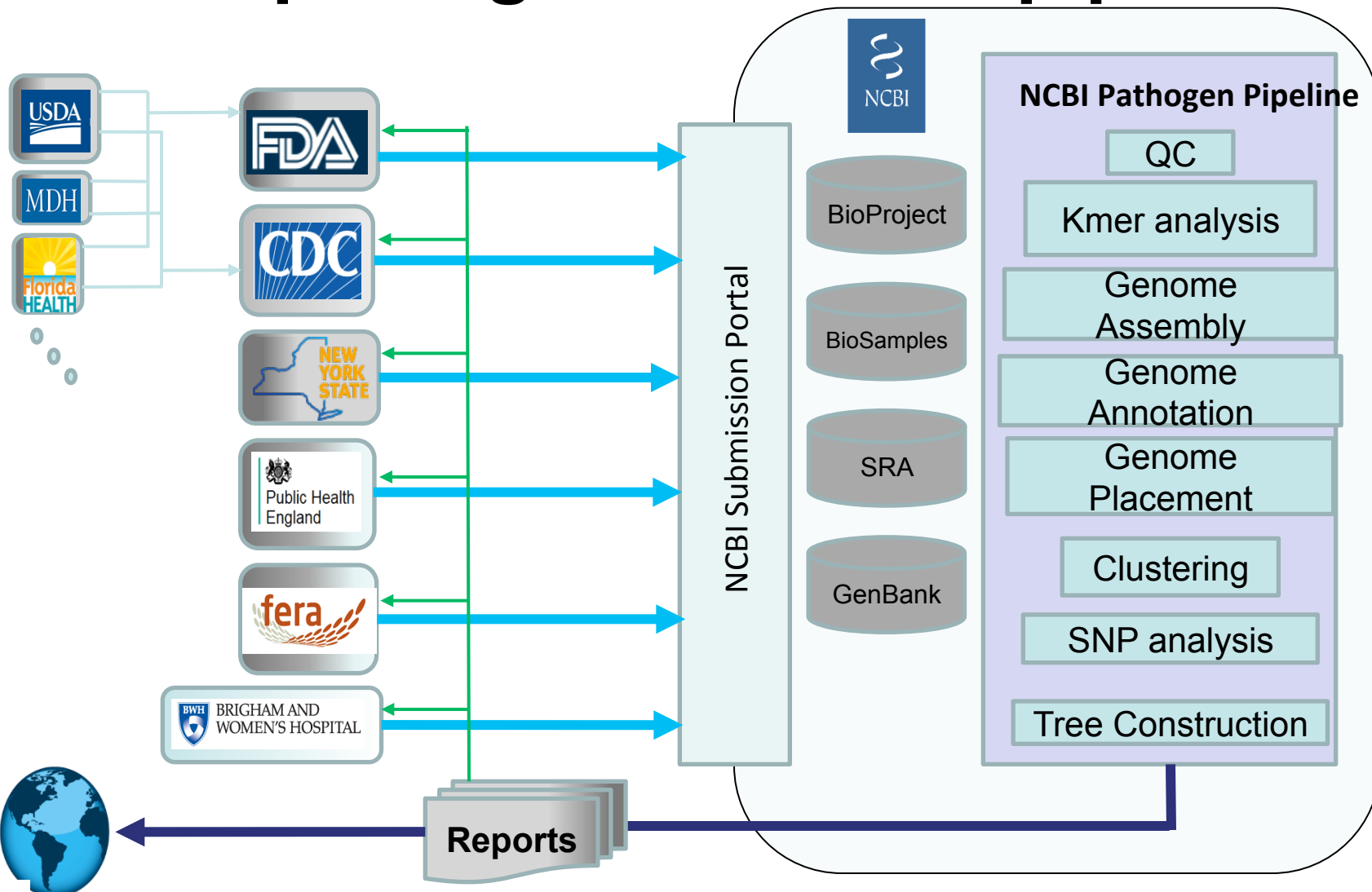


# Minimal pathogen metadata

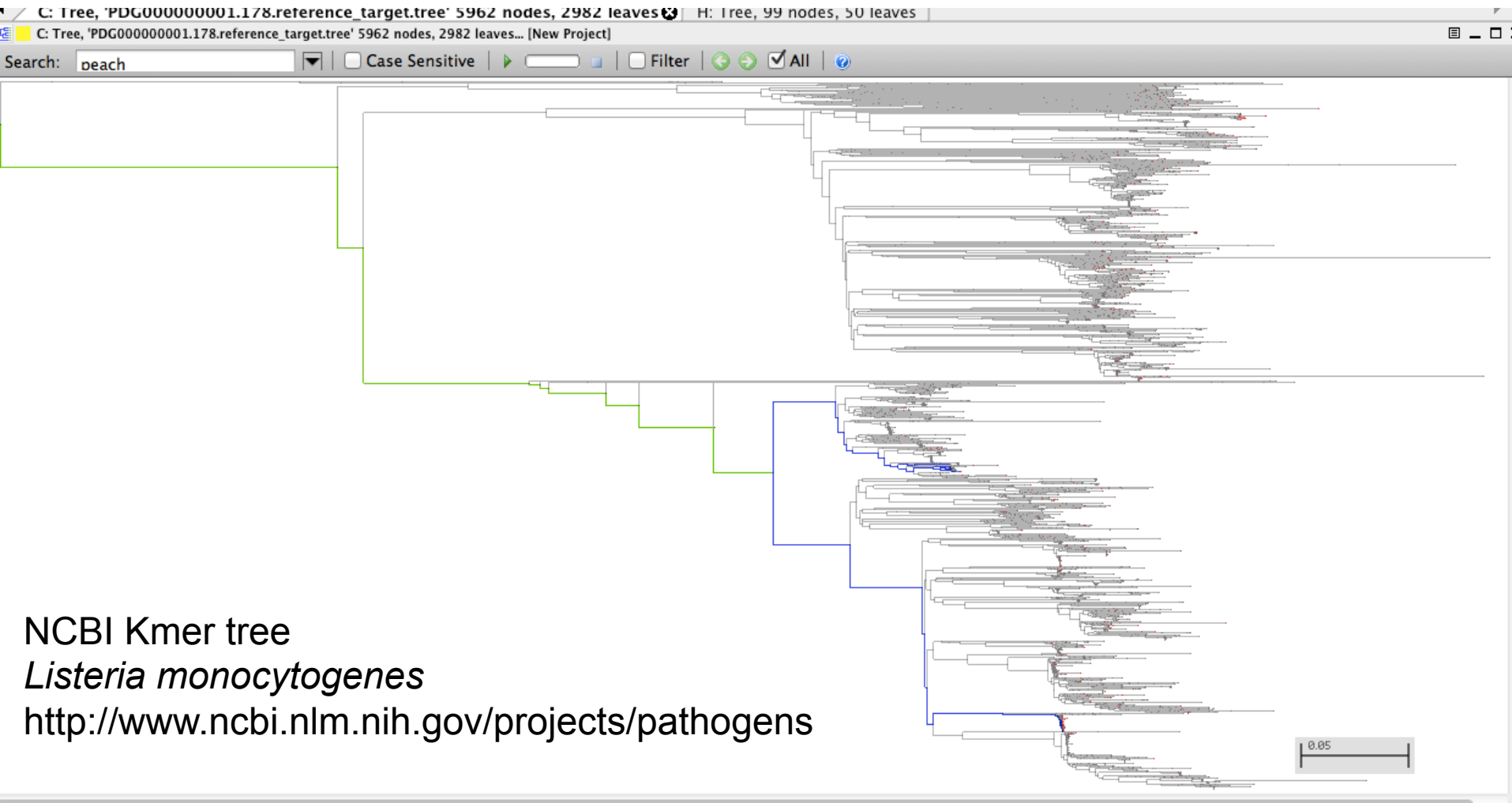
<p><b>sample_name</b> <b>organism</b> <b>strain/isolate</b></p> <p><u><b>What</b></u></p> <p>Category (attribute_package) 1a) <b>Clinical/Host-associated</b>     1a1) <b>specific_host</b>     1a2) <b>isolation_source</b>     1a3) <b>host-disease</b>     OR 1b) <b>Environmental/Food/Other</b>     1b1) <b>isolation_source</b></p>	<p><b>collection_date</b> <u><b>When</b></u></p>
	<p><b>Geographic location</b> <u><b>Where</b></u></p> <div data-bbox="908 699 1410 928"> <p>6a) <b>geo_loc_name</b> OR 6b) <b>lat_lon</b></p> </div>
	<p><b>collected by</b> <u><b>Who</b></u></p> <div data-bbox="1062 1028 1468 1170">  </div>



# NCBI pathogen detection pipeline



# Daily phylogenetic trees



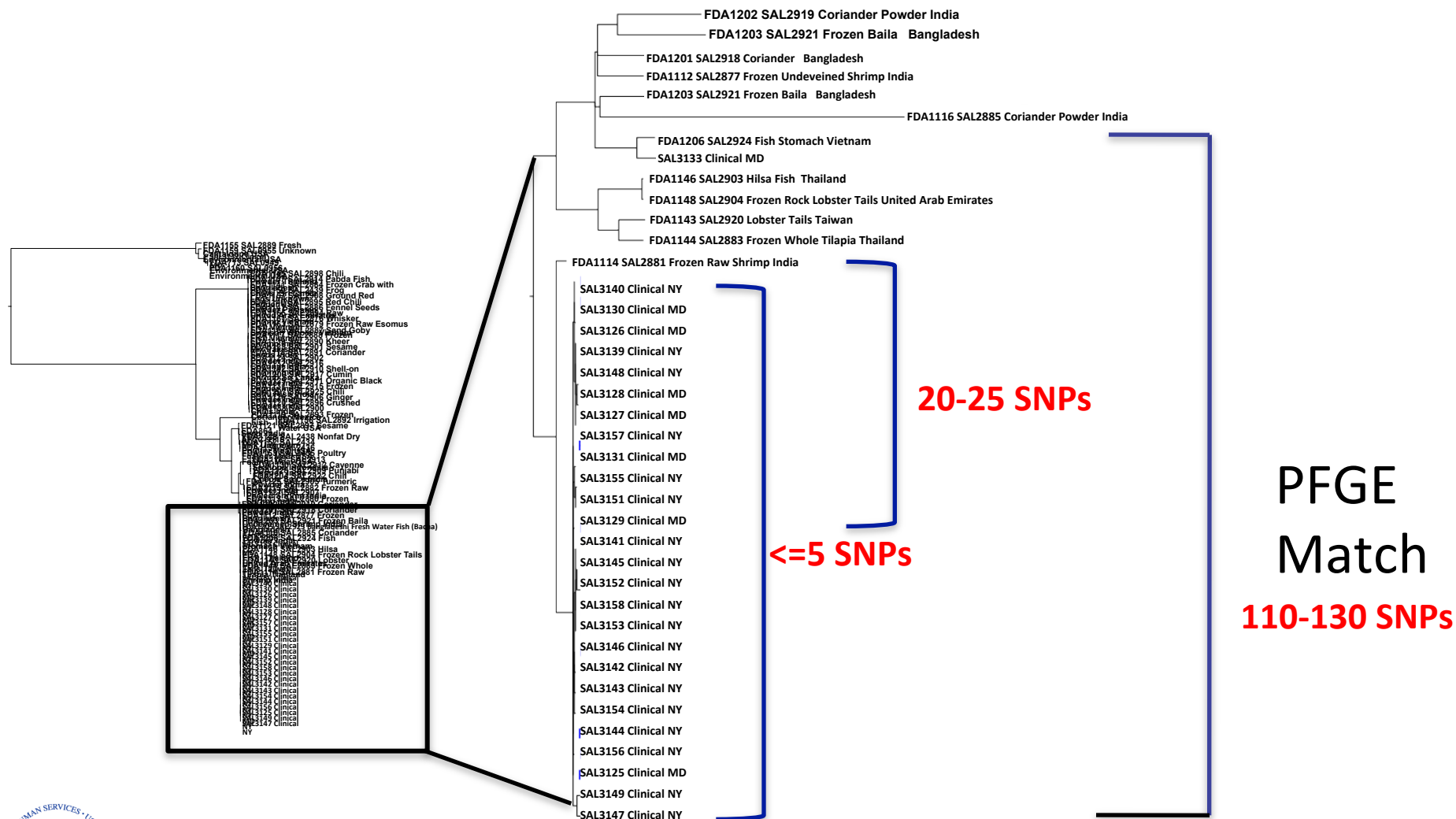
# Example 1:

## Global source traceback

- In 2012, 410 individuals in the U.S. became sick from ingesting food that contained *S. Bareilly*
- CDC investigated a multistate (29 states) outbreak
- The illnesses were linked to frozen raw yellowfin tuna (Nakaochi Scrape) which was imported from India
- FDA examined the WGS of these isolates and compared them to previous *S. Bareilly* events



# S. Bareilly Phylogeny







## NGS distinguishes geographical structure among closely related *Salmonella* Bareilly strains





# WGS supports preventative controls

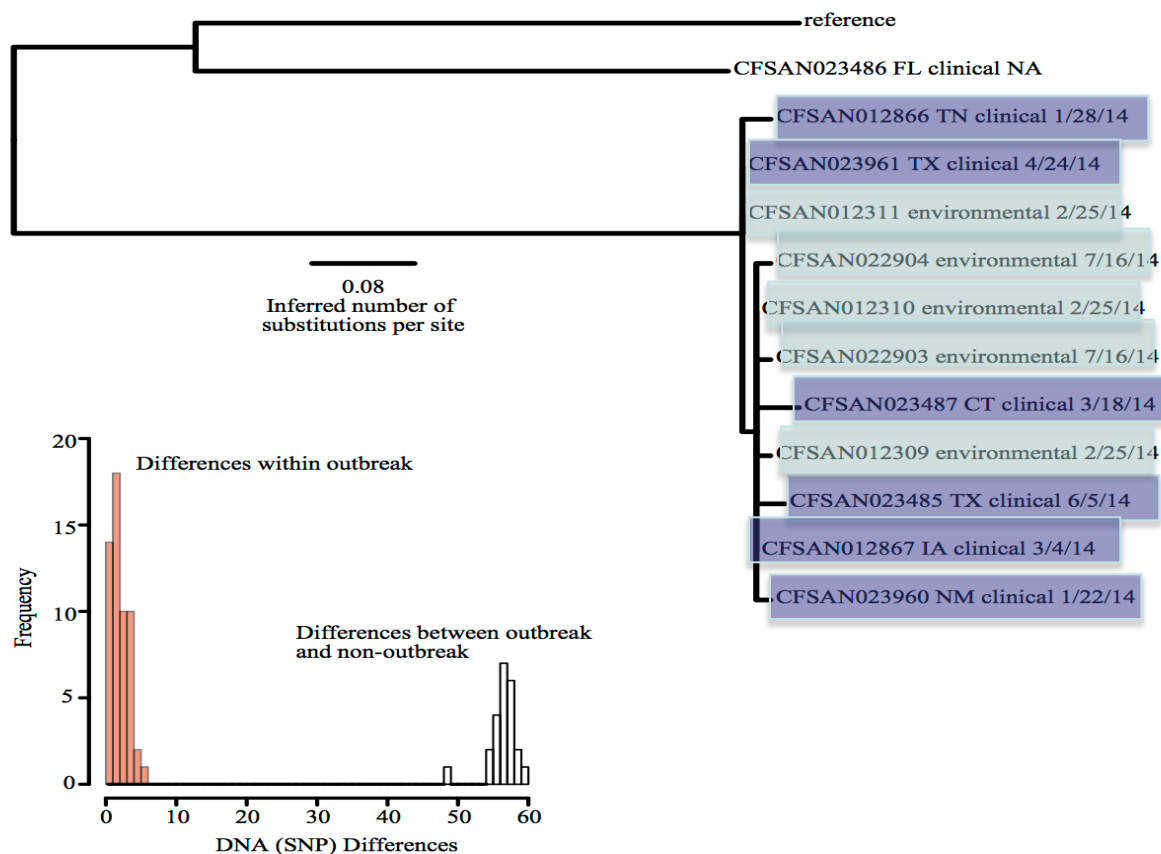
- Permits deep dive to solve persistent/complex problems in a facility or on a farm
- Comparison of internal WGS results to public database of food/environmental isolates
- Public software and analysis tools readily available to industry for viewing of results

## Example 2:

# S. Braenderup 2014 pre-outbreak

- In 2014, FDA conducted baseline environmental sampling in nut butter processing facilities
- A few of the samples tested positive for S. Braenderup and a PFGE pattern matched several cases of recent salmonellosis without a common link
- WGS was performed on both environmental and clinical isolates and found to be extremely close (2 SNP differences)

# S. Braenderup 2014 pre-outbreak



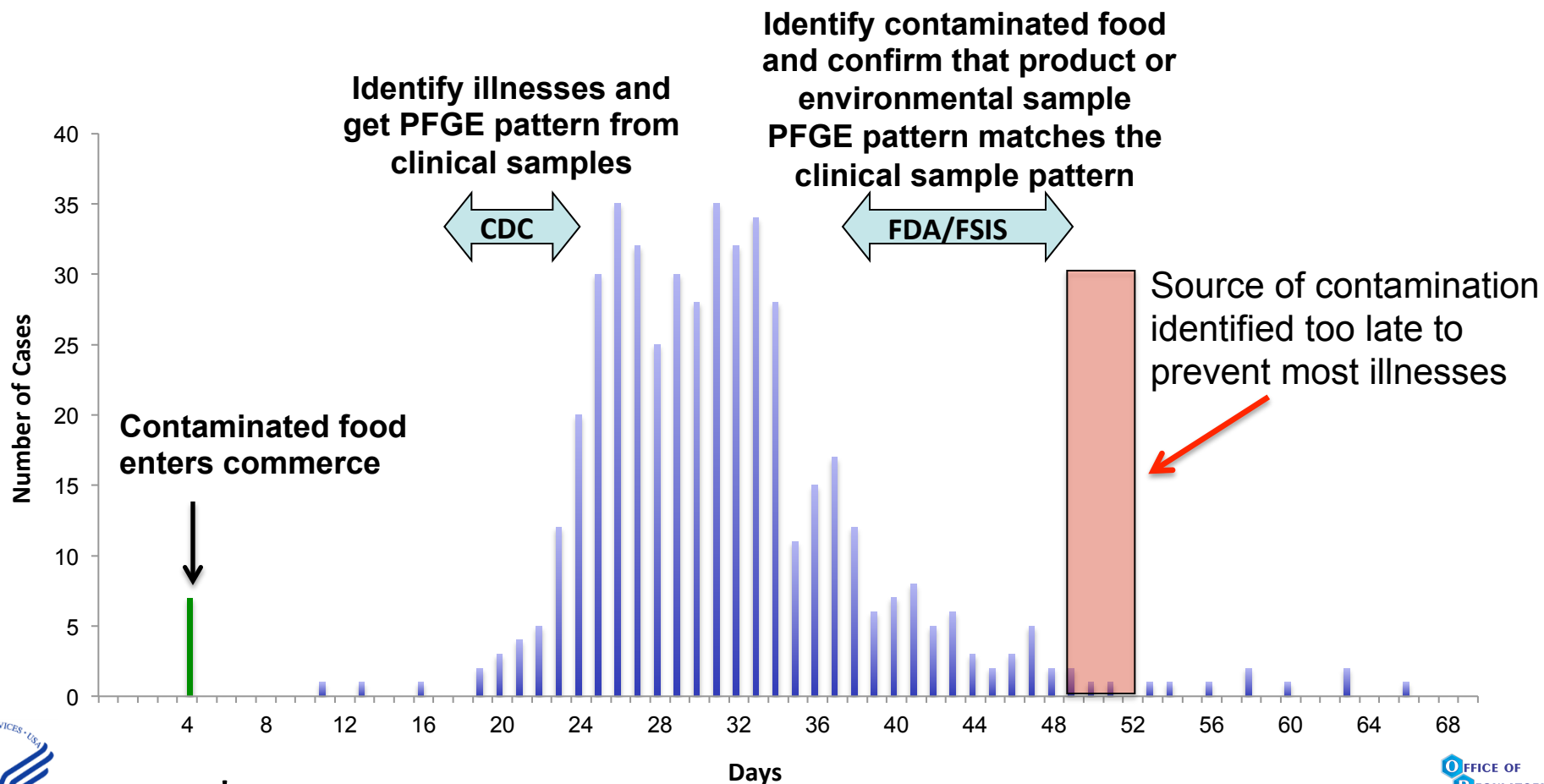
env. swab

clinical



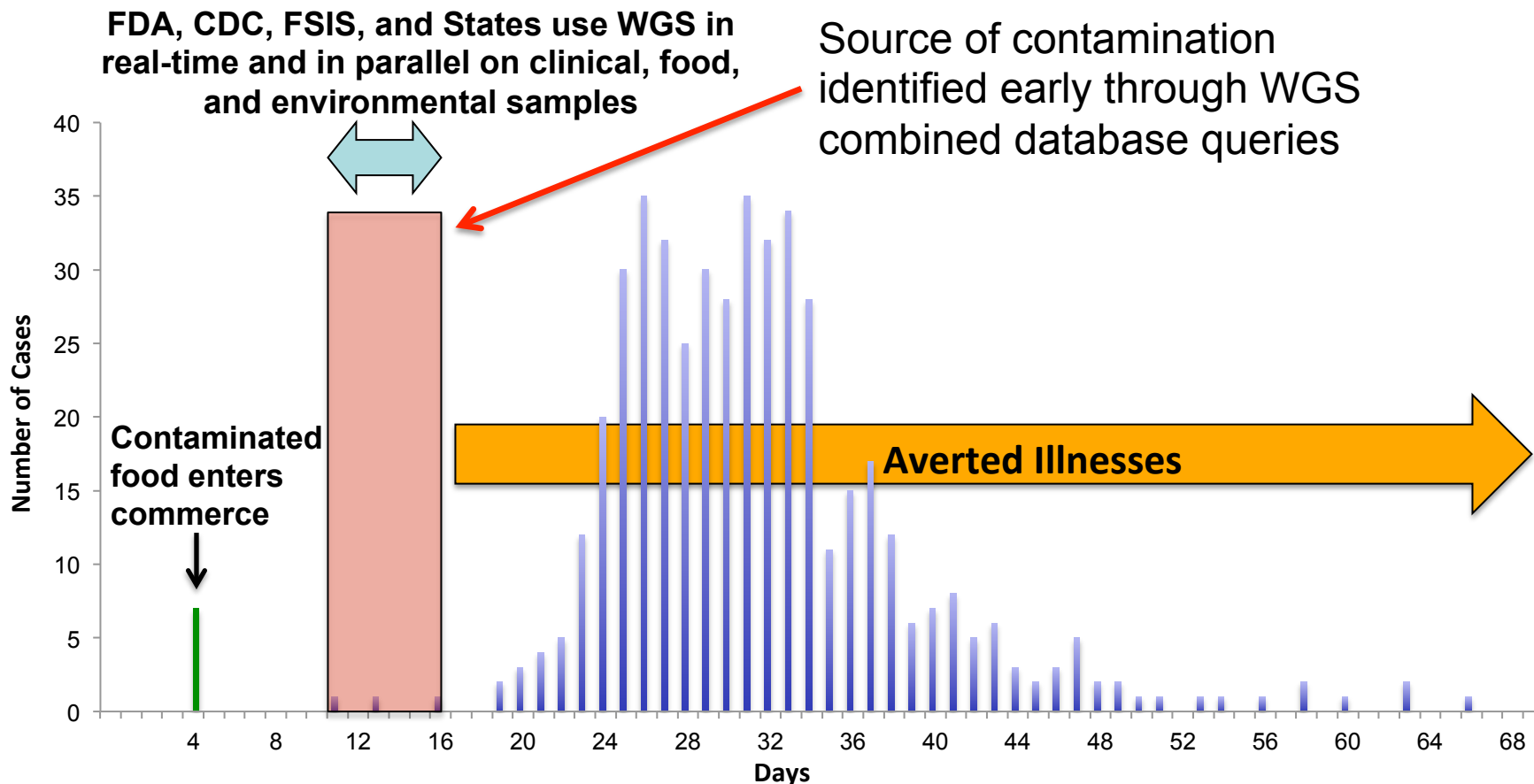


# Representative\* timeline for traditional approach (PFGE)



\* Data is for illustrational purposes and does not represent an actual outbreak

# Representative\* timeline using WGS



\*Data is for illustrational purposes and does not represent an actual outbreak

# **Immediate impacts of WGS to industry, growers, and distributors, 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



## 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 “Carlos 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**
- **Other independent collaborators**