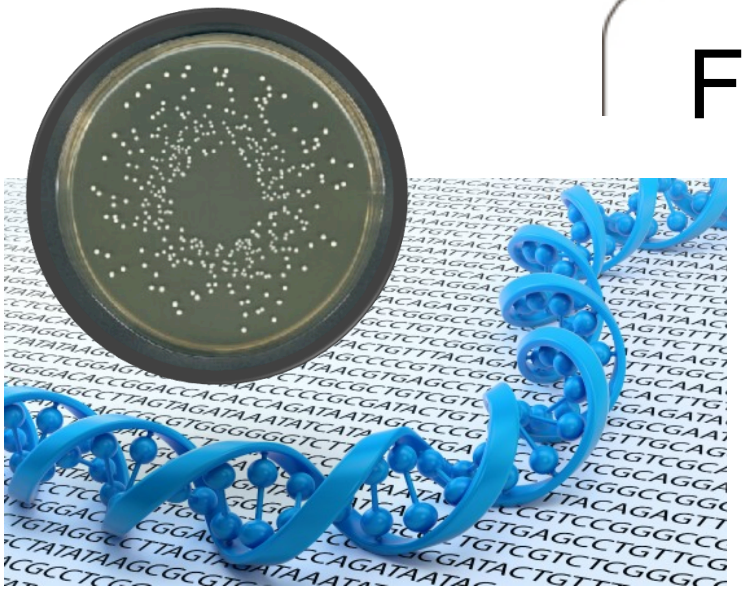


From Petri to Infinity

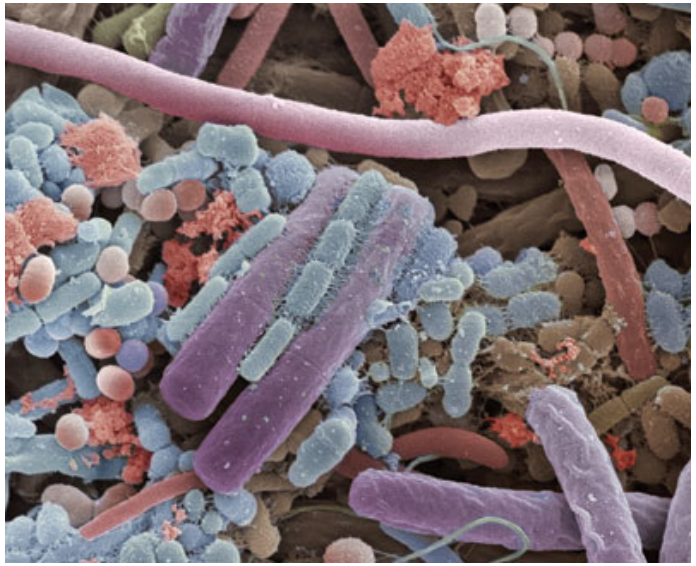
April 5, 2018

Ontario Food Protection Association Spring Technical Meeting

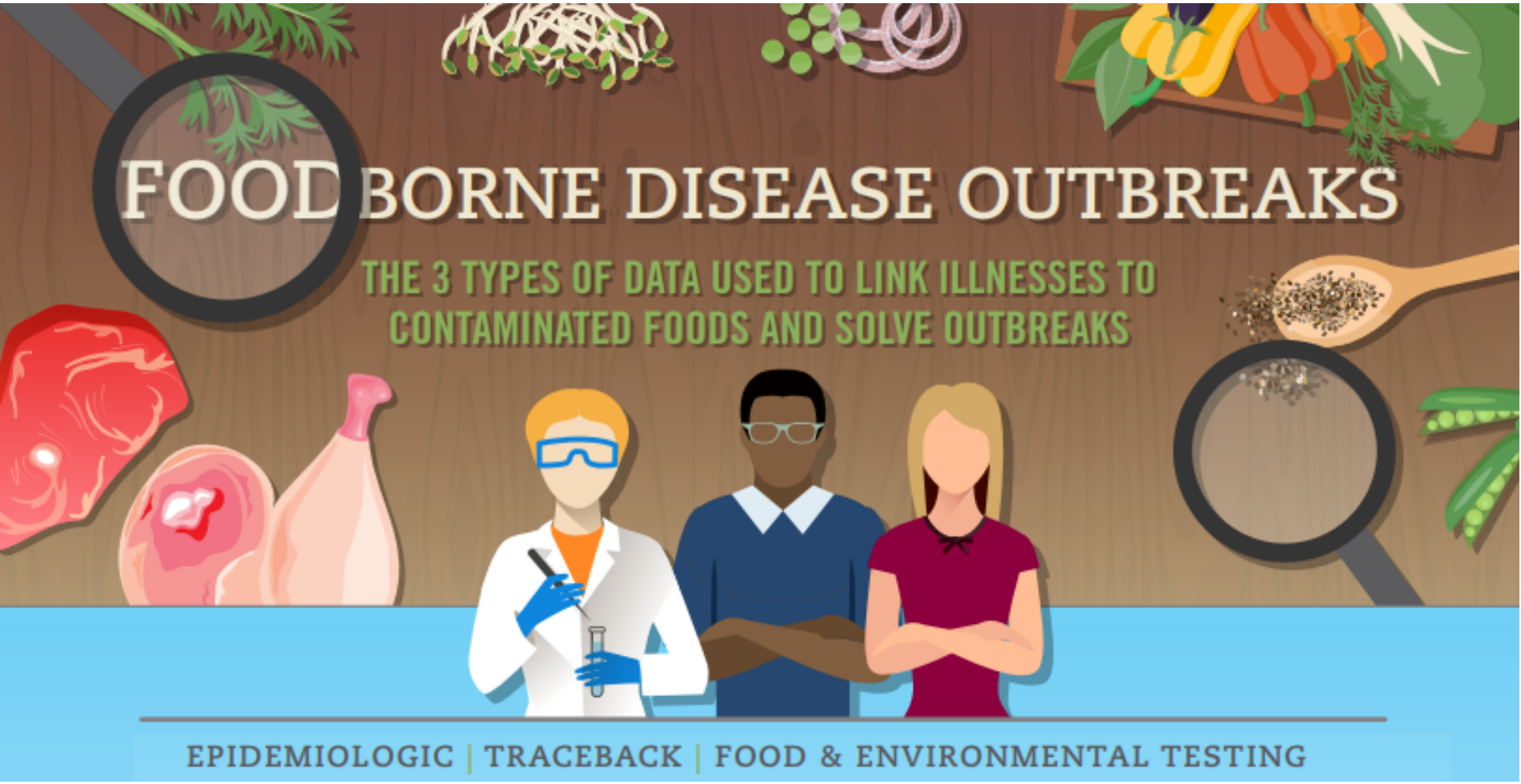
Jeanine Boulter-Bitzer, Ph.D.
Microbial Food Safety Science Analyst
Food Safety Science Unit
Ontario Ministry of Agriculture, Food and Rural Affairs

Outline

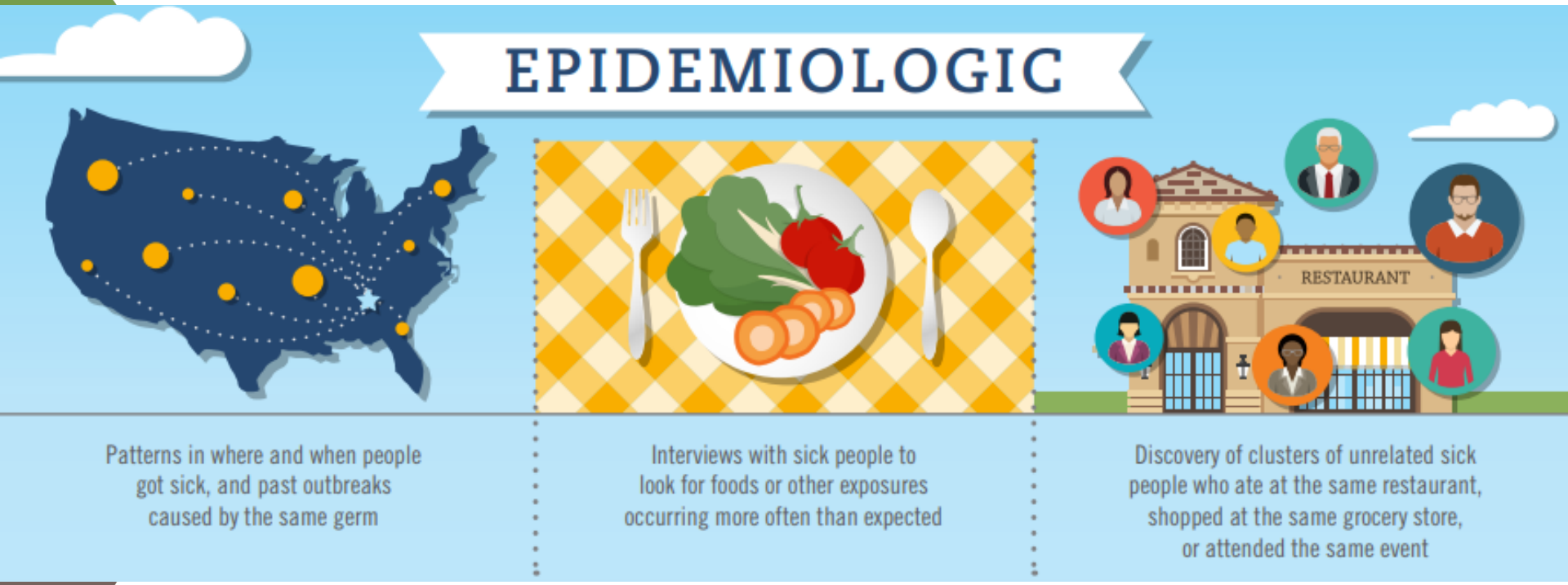
- Evolving detection methods for pathogenic bacteria & investigating foodborne outbreaks



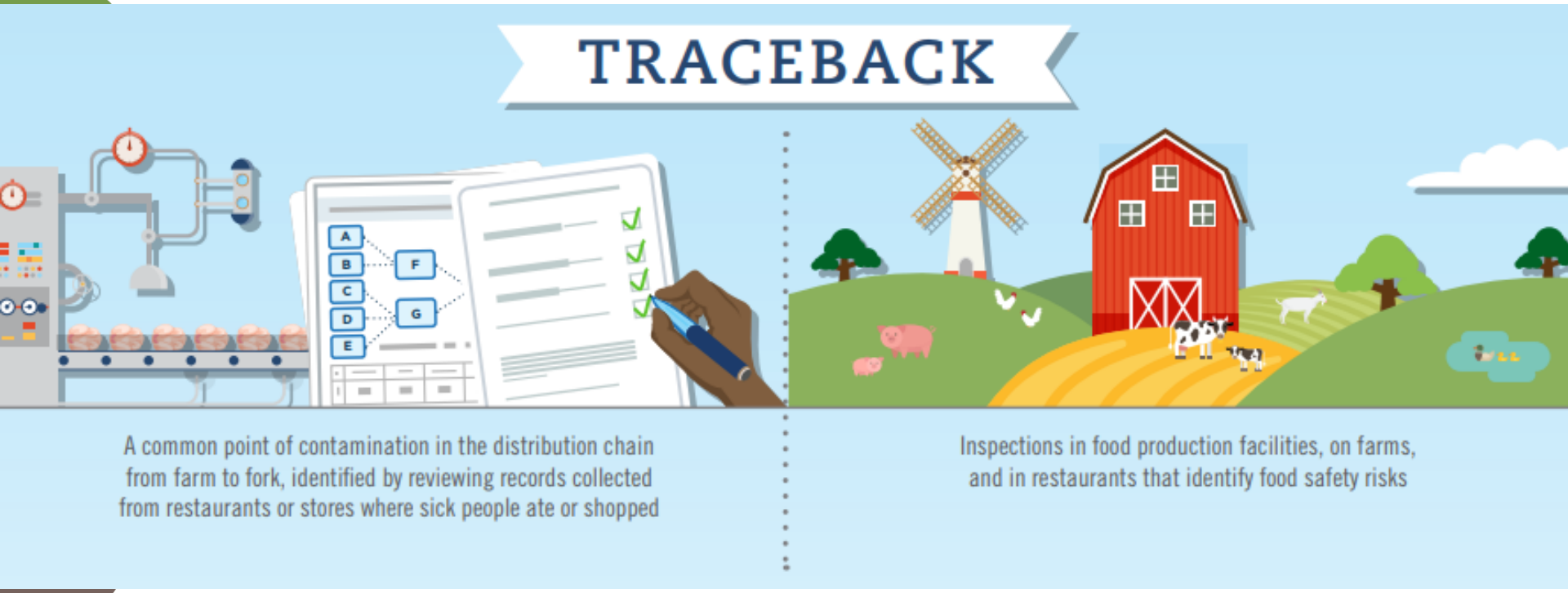
Outbreak investigation



Outbreak investigation



Outbreak investigation



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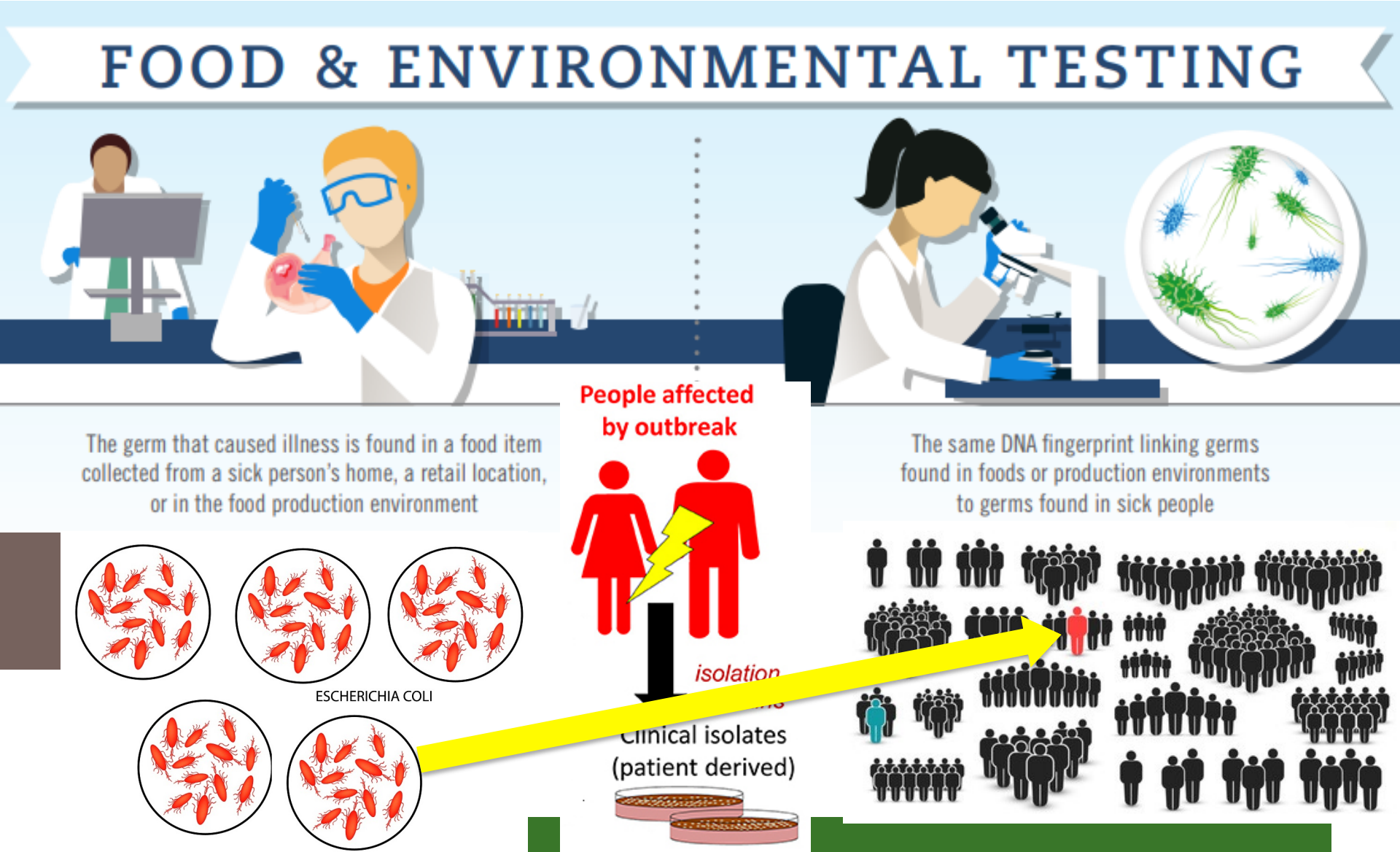
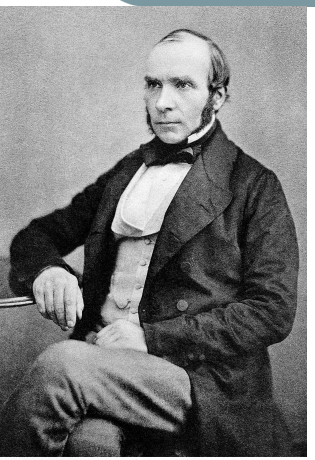


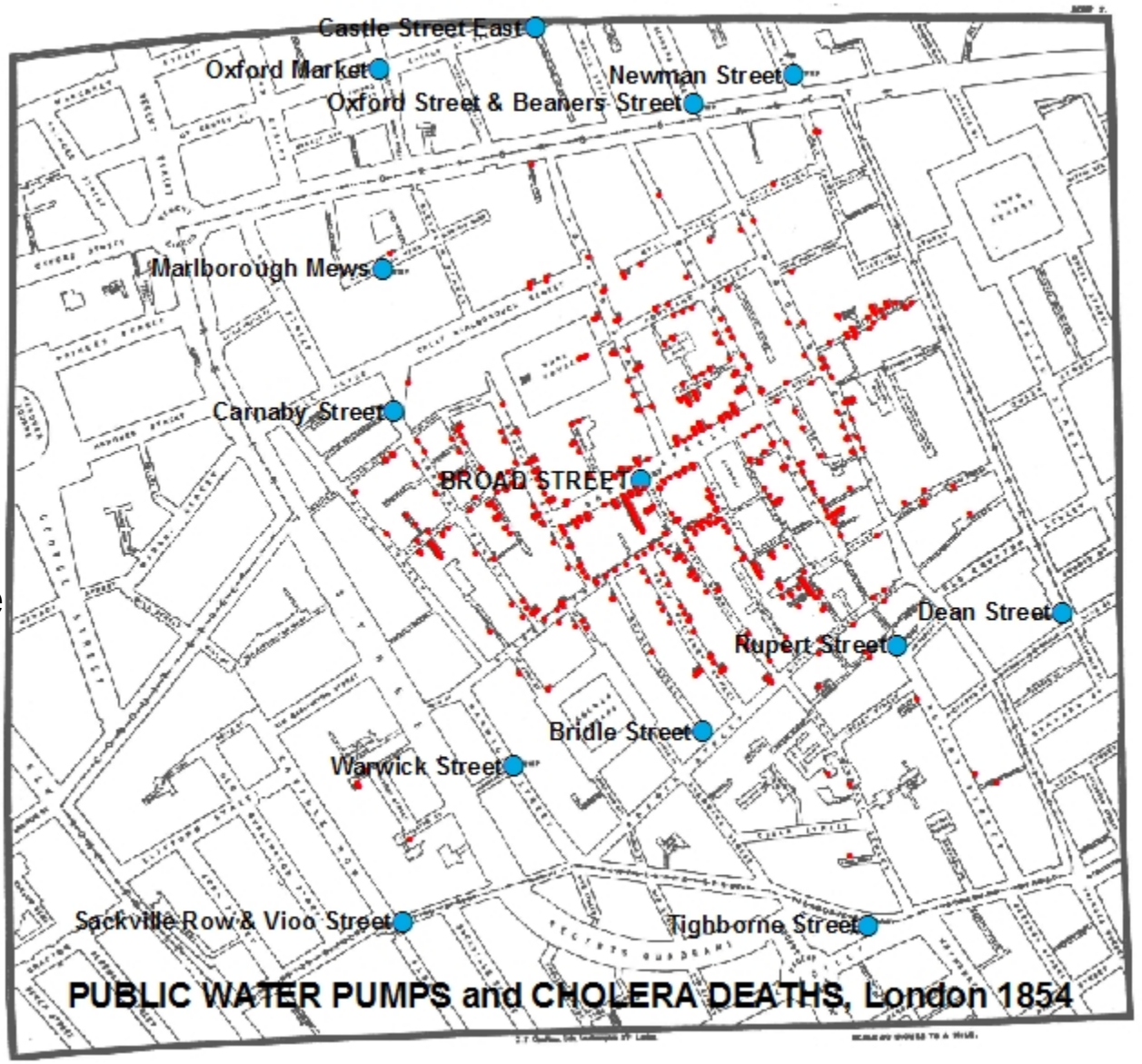
Epidemiology, 1854



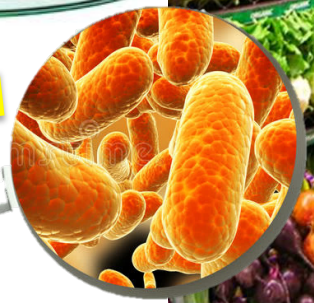
Dr. John Snow

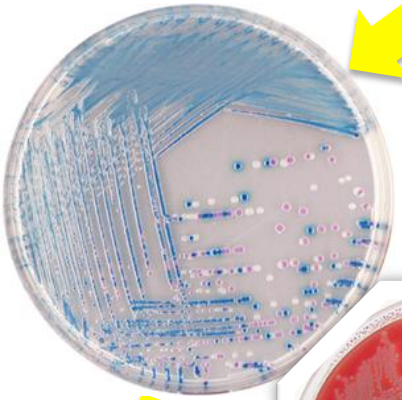
- Theorized deaths spread via contaminated water.
- Microscopically confirmed the presence of an unknown bacterium in the Broad Street pump samples.

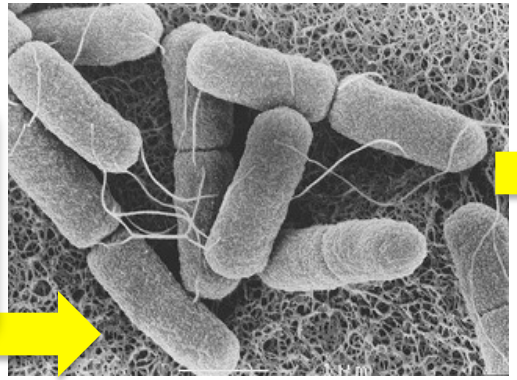
578 cholera deaths, (*Vibrio cholera*)

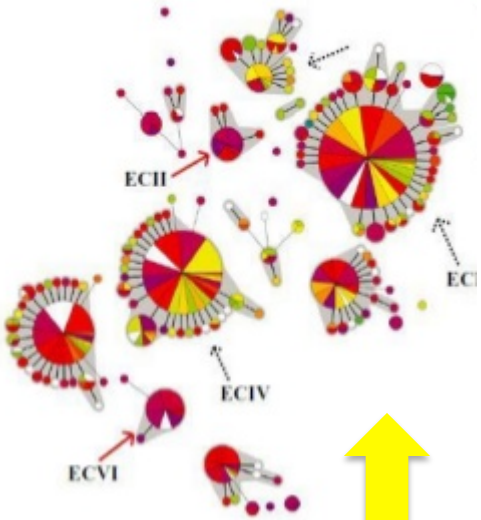


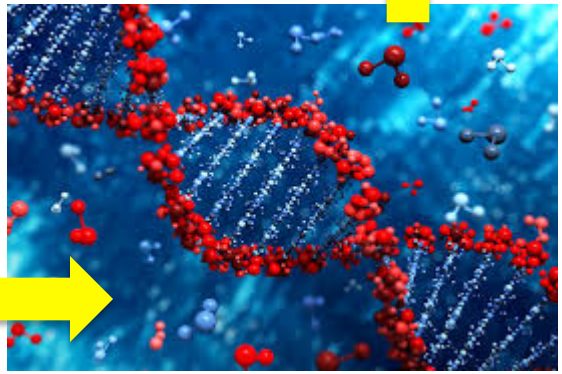
Bacterial Culture





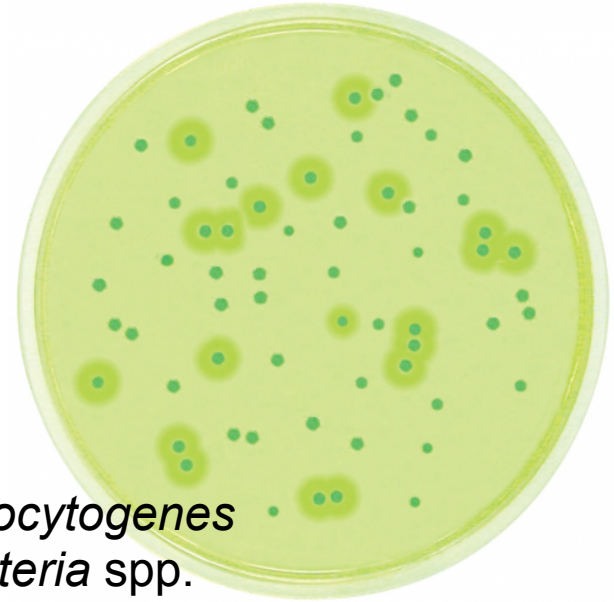




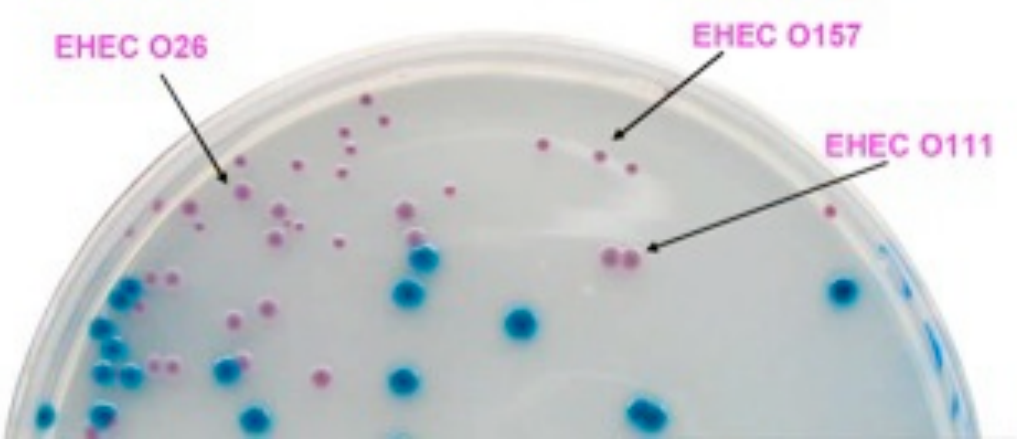


Culture to ID pathogens in food

- Detection of foodborne pathogens has been conducted via culture-dependent techniques (still considered the gold standard).
- Growth of viable organism
  - Then: biochemical tests, subtyping, sequencing…
- Some culture for pathogens is excellent, for others, difficult.



L. monocytogenes and *Listeria* spp.

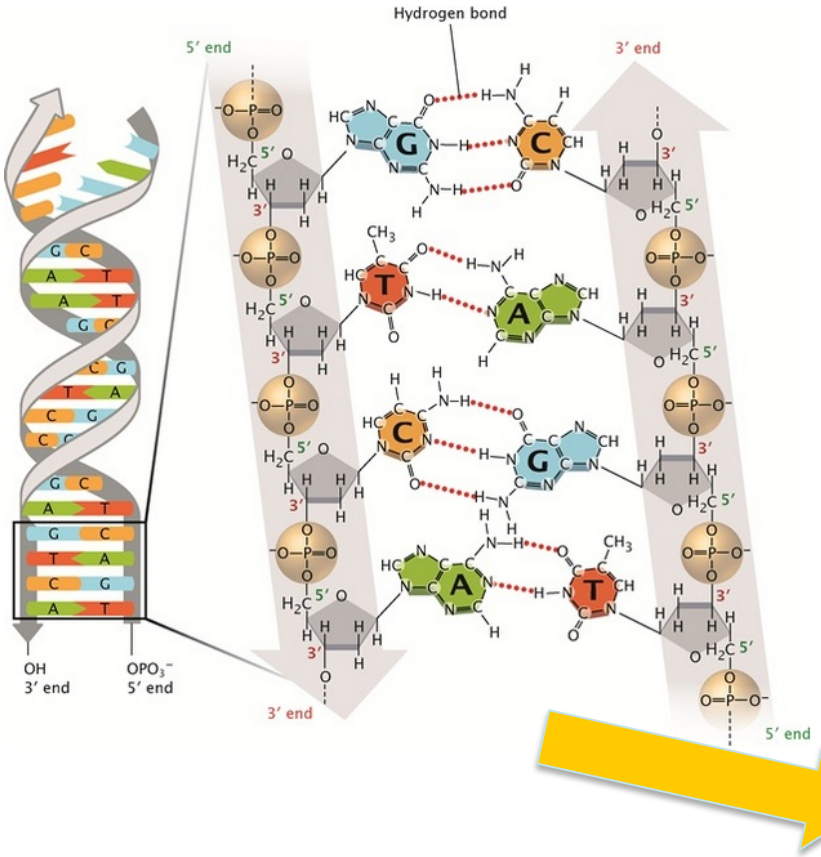


STEC

Culture to ID pathogens in food

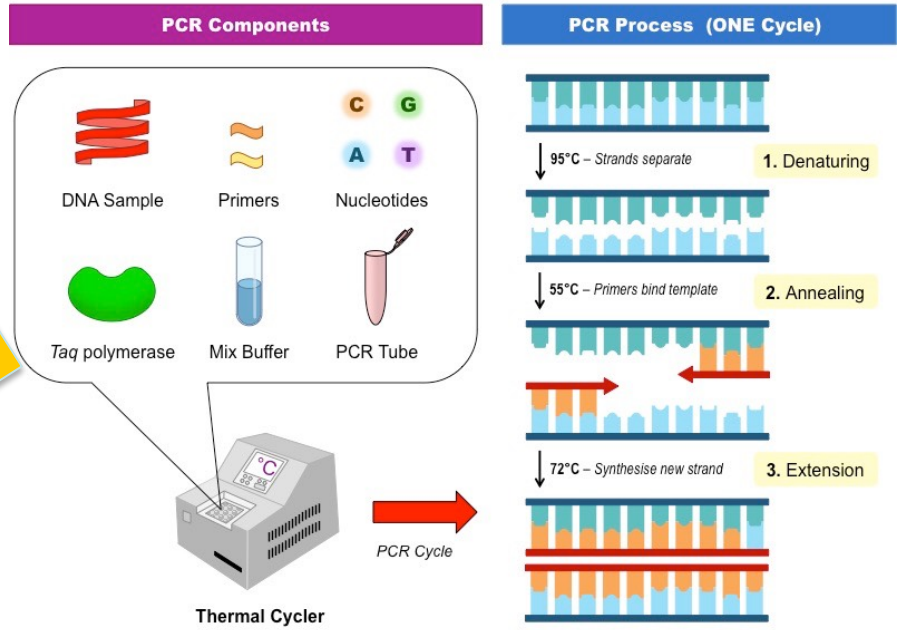
- Hazards with culturing:
  - Heterogeneous distribution in food
  - For some of the highly infectious bacterial pathogens, the concentration in outbreak associated product may be below 1 cell per 25g of product.
    - Without enrichment, there are few to no technologies that can approach this sensitivity.
    - Minimum time required for sample analysis is determined by the enrichment period (6h, 24h, etc.); time required to reach the LOD (the minimum concentration of cells that can be detected) of the method of analysis.
  - Confounded by processing technologies:
    - Reversibly injured= viable cells that can replicate following repair.
    - Irreversibly injured= cells which can not replicate but may retain metabolic activity.

Beyond identification: Typing



- DNA was first identified in the late 1860s by Swiss chemist Friedrich Miescher.
- Publication of DNA Structure and Function: Watson and Crick, 1953.

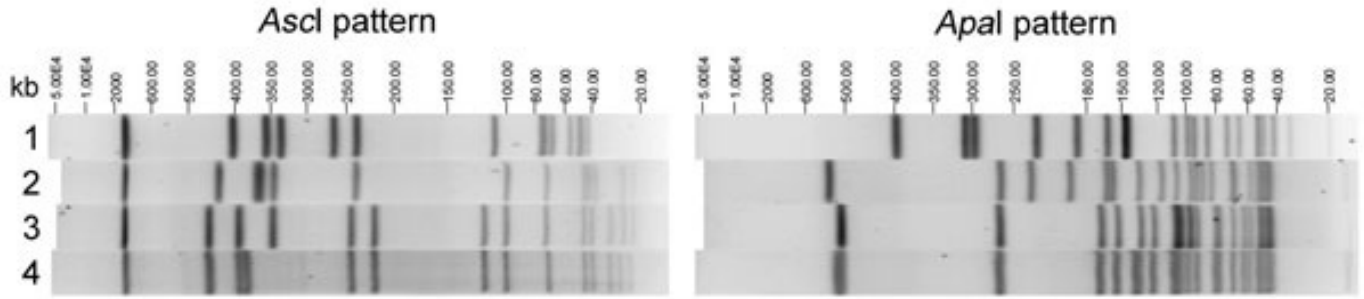
- Polymerase Chain Reaction (PCR) technique, invented in 1985 by Kary B. Mullis: copy DNA.



Beyond identification: Typing

- Genomic analysis to ID isolates to the genus or species level by detection of specific gene markers by PCR-based has greater discrimination than phenotypic methods. Can link isolates from clinical, food and environmental samples.
  - Outbreak identification, source tracking.
- PFGE

  1984

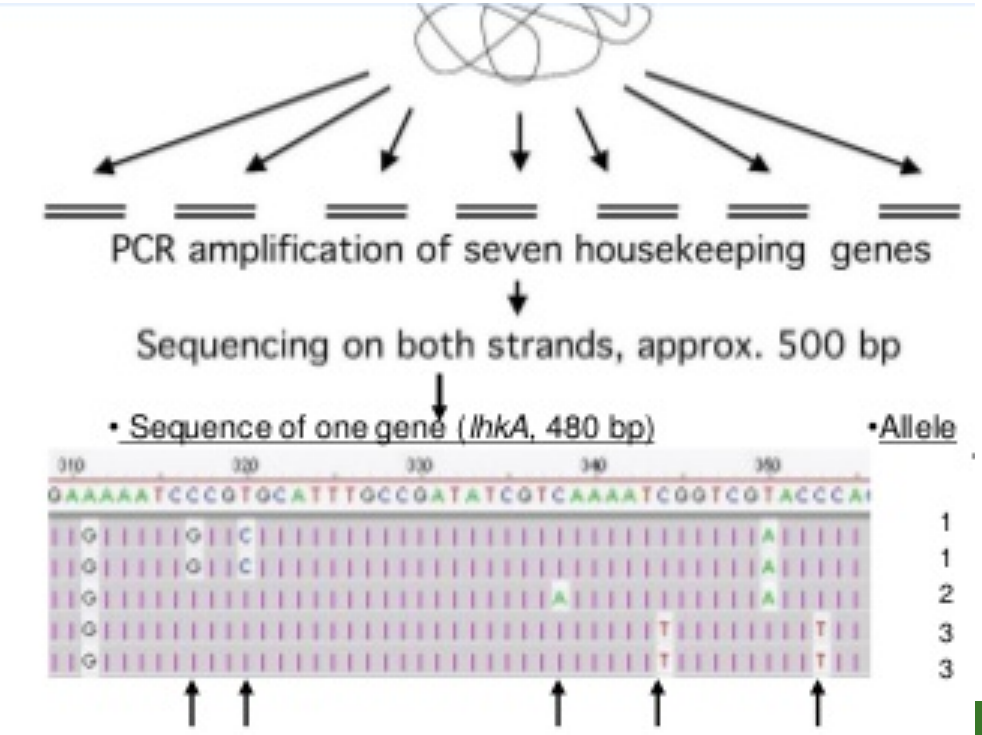


  - Genome insertions, deletions, rearrangement, and point mutations at a restriction enzyme site can cause Lm isolates that are highly related genetically to appear different by PFGE.
  - However, isolates that are not highly related may appear indistinguishable by PFGE

Beyond identification: Typing

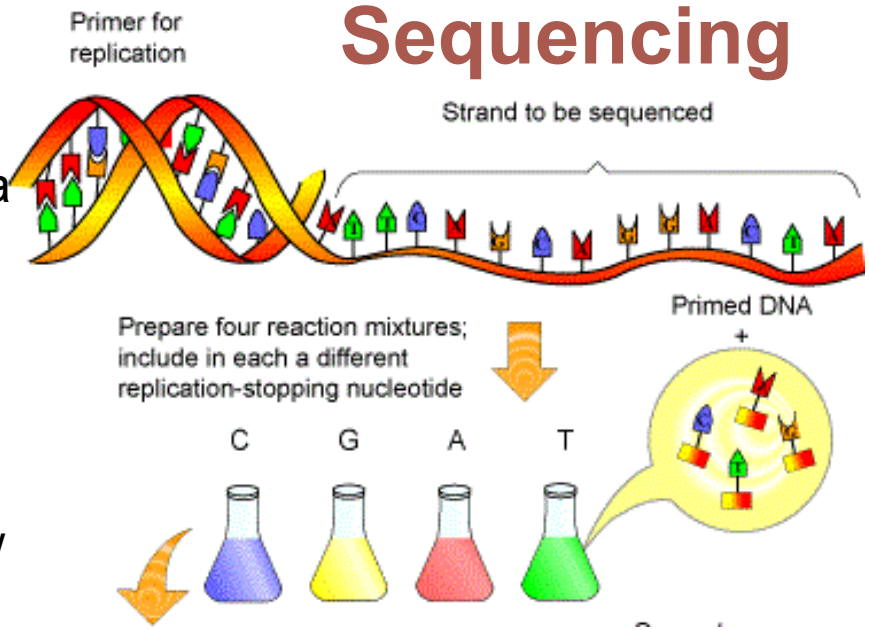
- Multilocus sequence typing (MLST) analyzes DNA sequences of internal fragments of housekeeping genes.
  - 400 – 500bp gene fragments are sequenced.
  - The method looks at variations in these DNA sequences between strains.
  - The relatedness of isolates are made by comparing allelic profiles.

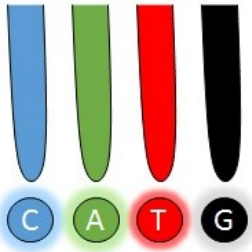
1998



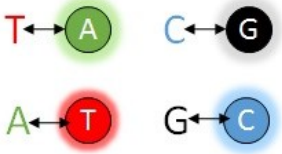
Sequencing

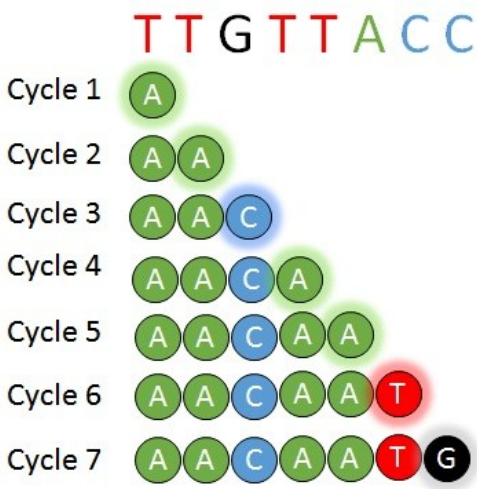
- DNA sequencing is the process of determining the sequence of nucleotides (As, Ts, Cs, and Gs) in a piece of DNA.
- In Sanger sequencing, the target DNA is copied many times, making fragments of different lengths. Fluorescent “chain terminator” nucleotides mark the ends and allow the sequence to be determined.

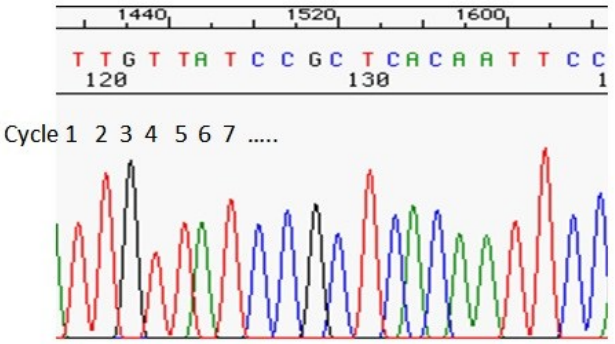




Nucleotides that glow are used to figure out the “sequence” of nucleotides in a string of DNA







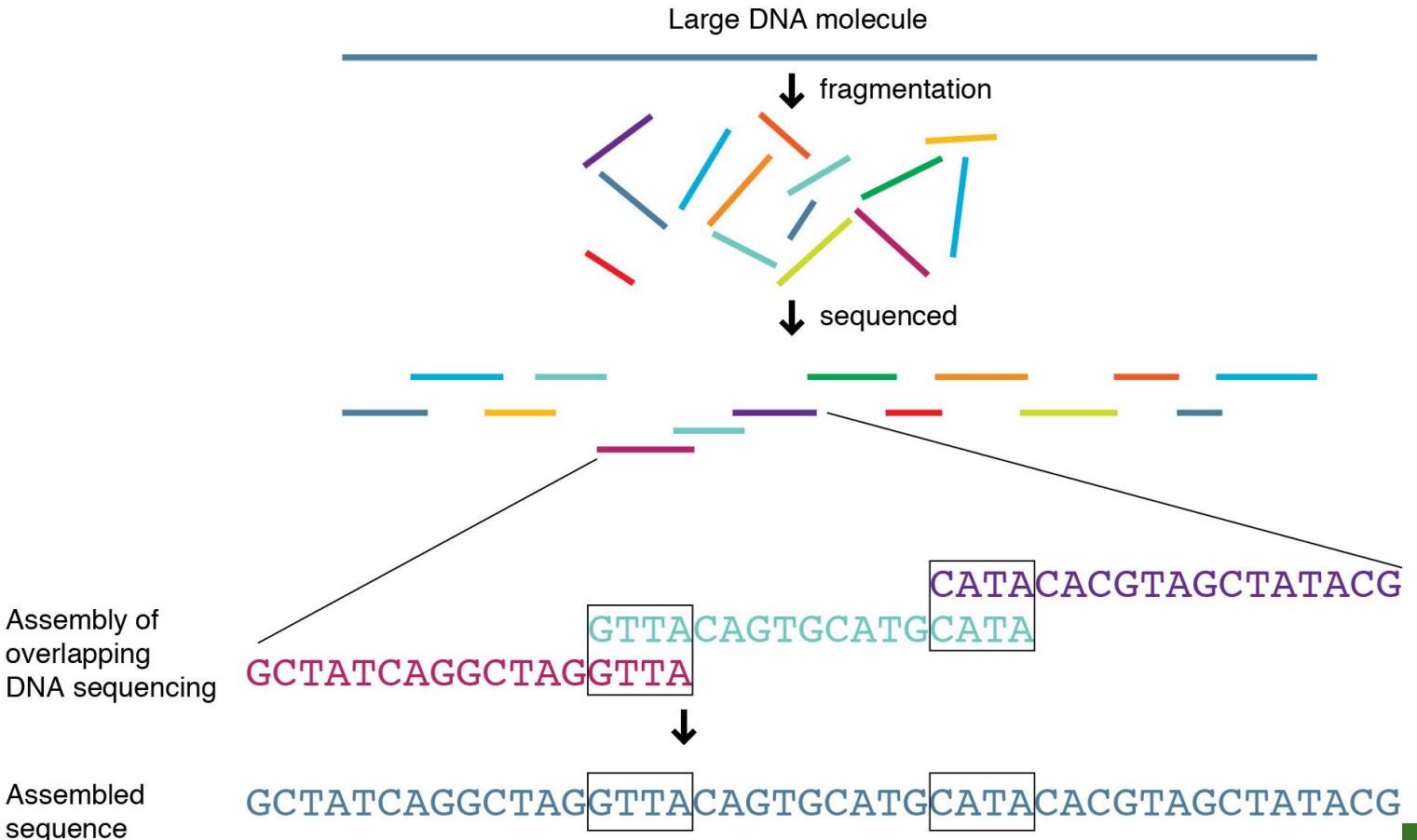
Glowing nucleotides are “read” one by one by a detector that makes and image like the one above that shows the “sequence” of the nucleotides

NGS: Next-Generation Sequencing

- Next-generation sequencing techniques are new, large-scale approaches that increase the speed and reduce the cost of DNA sequencing.
- Next-generation sequencing refers to non-Sanger-based high-throughput DNA sequencing technologies. Millions or billions of DNA strands can be sequenced in parallel, yielding substantially more throughput and minimizing the need for the fragment-cloning methods that are often used in Sanger sequencing of genomes.

WGS: Whole Genome Sequencing

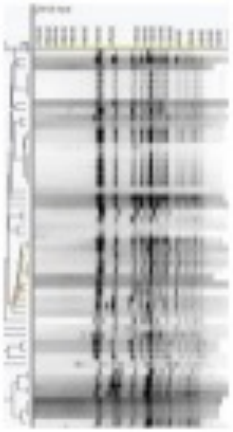
- Whole Genome Sequencing (WGS): replacement or compliment to molecular tests for ID, typing and characterizing pathogens.
- Complete sequence of all genomic content.



WGS: Whole Genome Sequencing

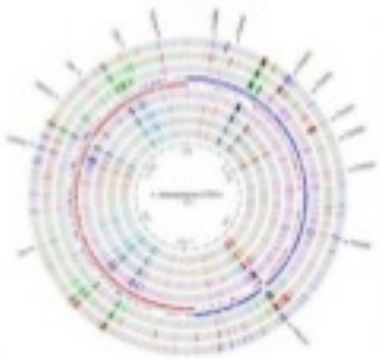
- Higher discriminatory power than conventional typing: PFGE, MLST, MLVA, phage typing, virulence testing…
    - E.g. *Salmonella enterica* Enteritidis are difficult to discriminate with other typing methods.
- Dependent (still) on the presence of isolates.
- WGS is recent in terms of use for isolate ID, subtyping, virulence determination, source tracking for decision-making.
    - Complex computational analysis is required. *E. coli* 4.6Mb, STEC 5.44Mb, *L. monocytogenes* 2.9Mb.
    - Standards for analysis and interpretation have yet to be established. E.g. AMR prediction from WGS (“‘poor’ but in development”)
- Currently, challenges in relating genomic data to phenotype must be recognized, and currently require culture.

Discrimination

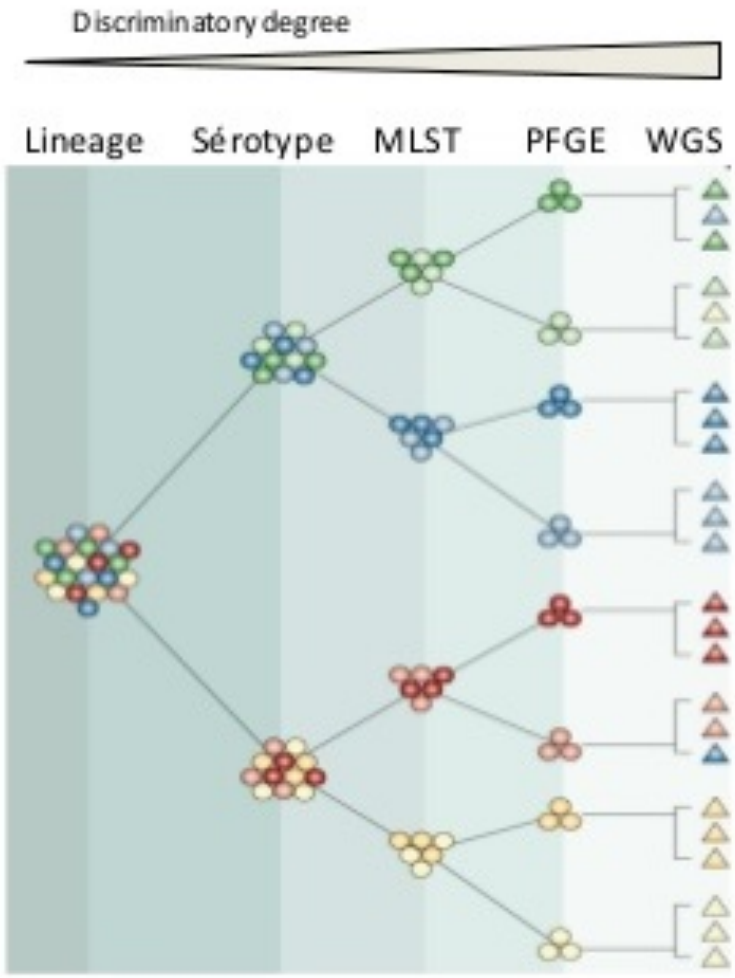


PFGE : The current international standard -Outbreak investigation

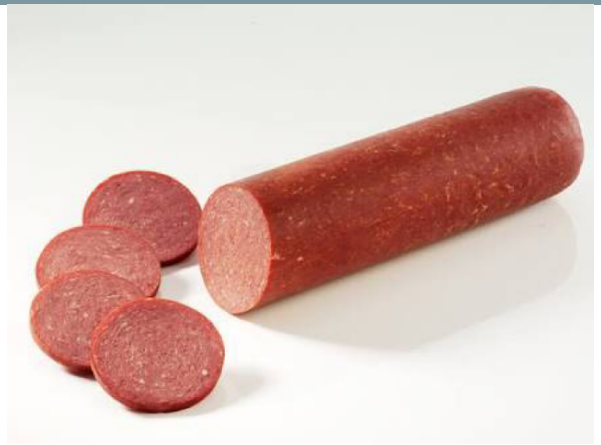
MLST : Global epidemiology
Population biology
Phylogeny

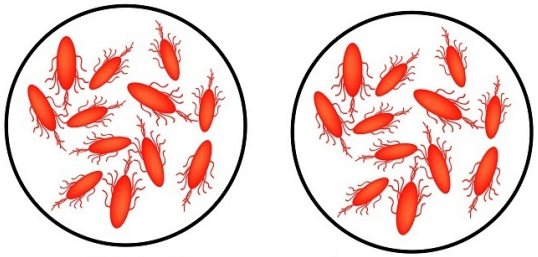


WGS : Information on pathogen genomes (species identification, drug resistance, virulence, evolution…).



Outbreak investigation





Listeria monocytogenes

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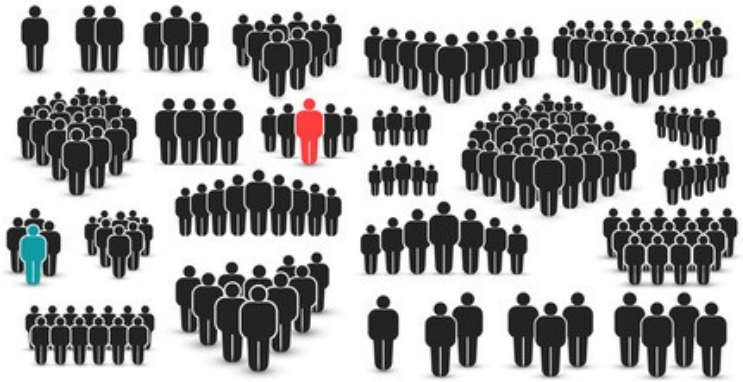
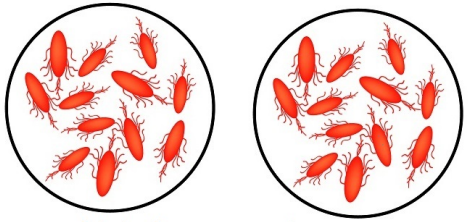
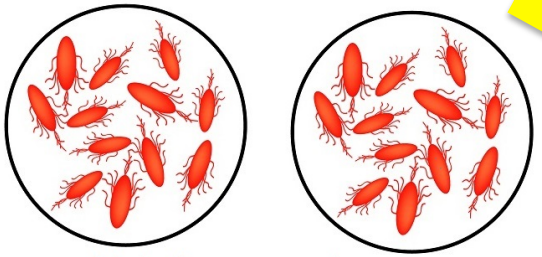




Listeria monocytogenes

Outbreak investigation

Where was this made?



Listeria monocytogenes

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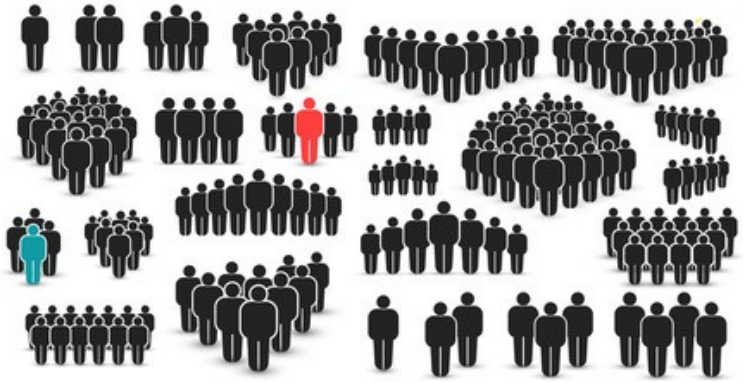
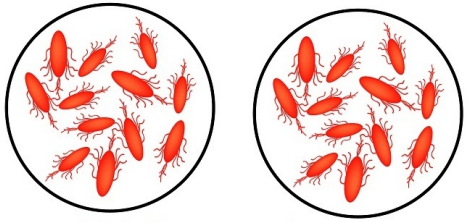
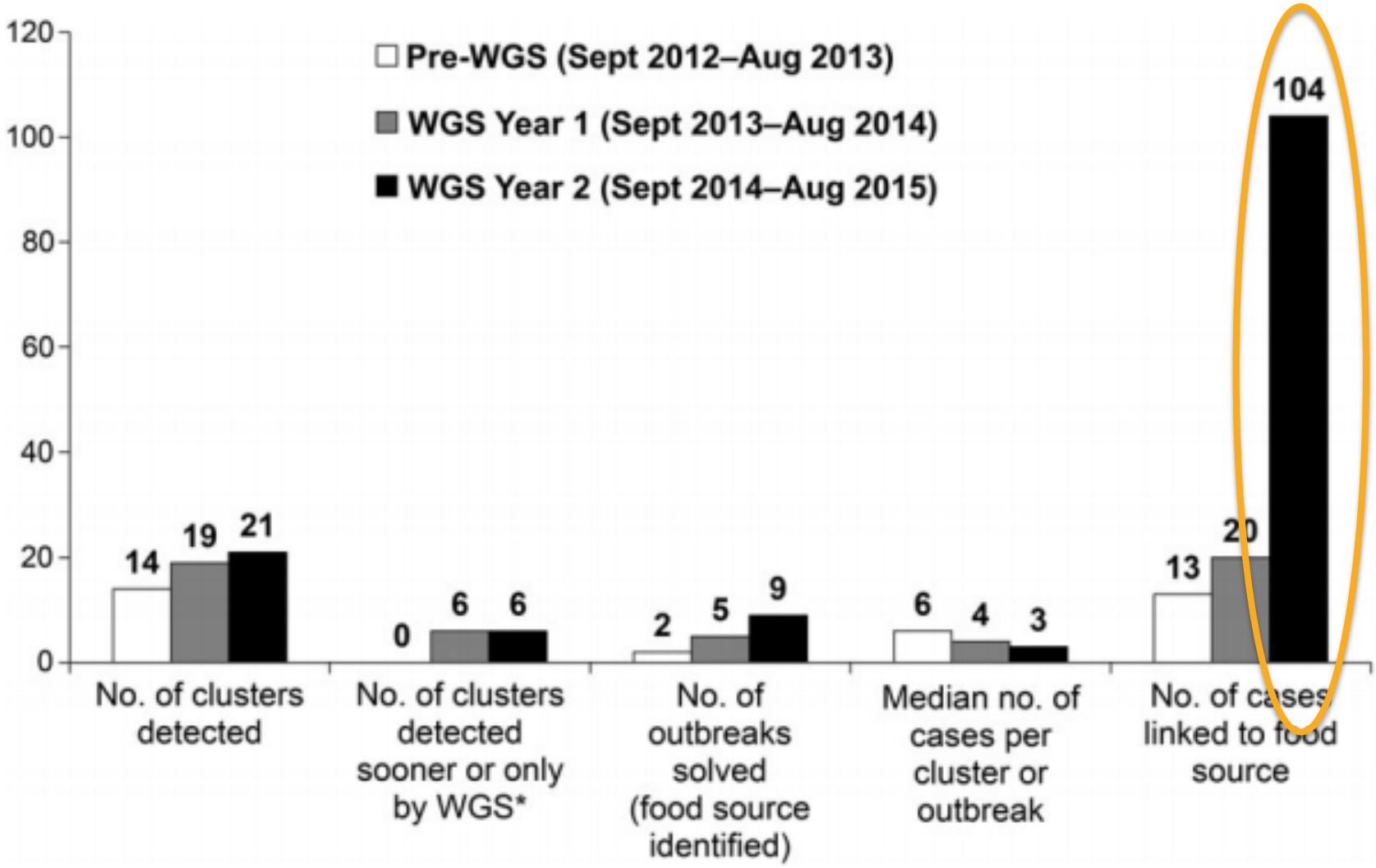




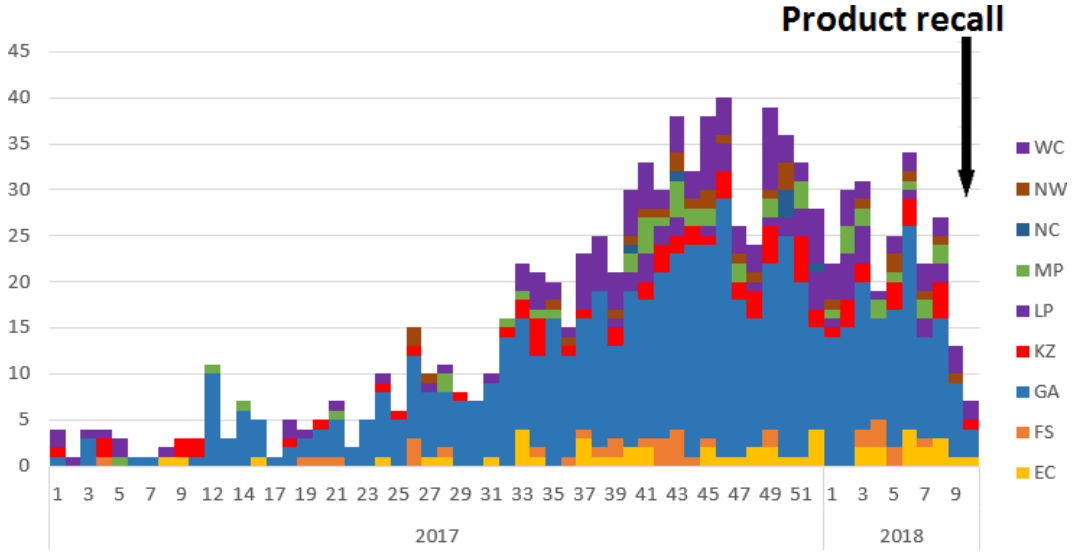
Listeria monocytogenes

WGS & Outbreak Detection



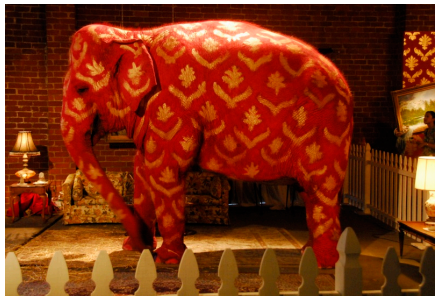
WGS & Listeriosis Outbreak S. Africa

- In South Africa, an outbreak of listeriosis, has had 978 laboratory-confirmed listeriosis cases from 1 Jan 2017 through 14 Mar 2018.
- Globally, this is the largest outbreak of listeriosis that has been detected, 42% of patients were neonates (infected during pregnancy).

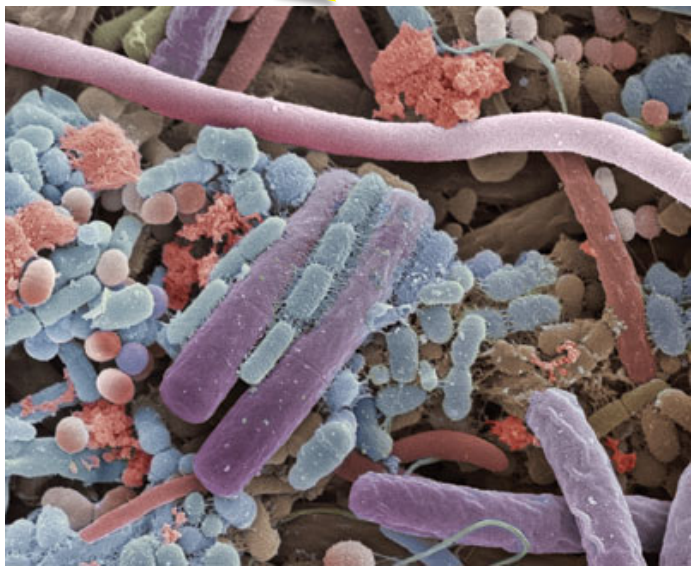


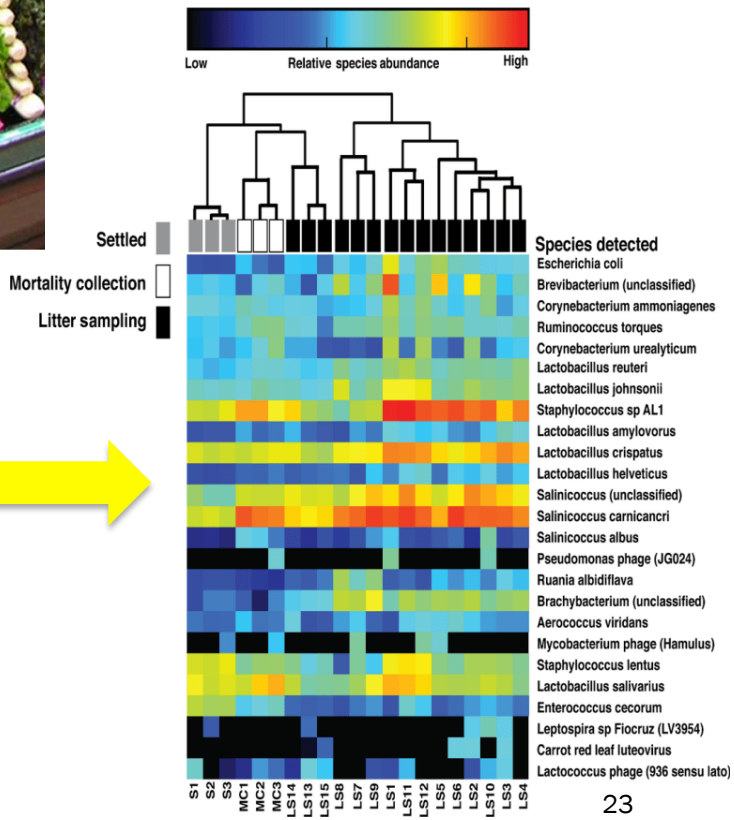
- The outcome of illness is known for 674 patients, of whom 183 (27%) of them died.
- Whole genome sequencing was performed on isolates from a large subset of patients.
  - 91% *Listeria monocytogenes* Sequence Type 6 (ST6).
- The same ST6 sequence type was identified in a RTE processed meat product called “Polony” (also found in the processing environment).
- On 4 March 2018 the Ministry of Health it was believed to be the source.

Where are we going?

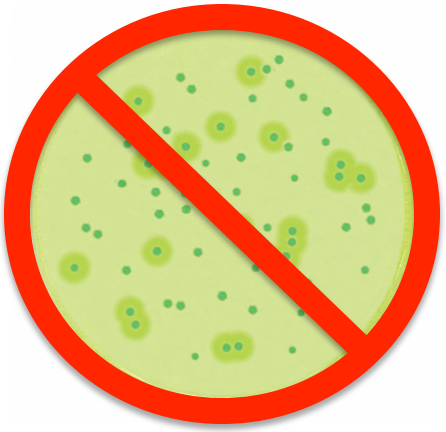


CIDTs: Culture-independent diagnostic tests





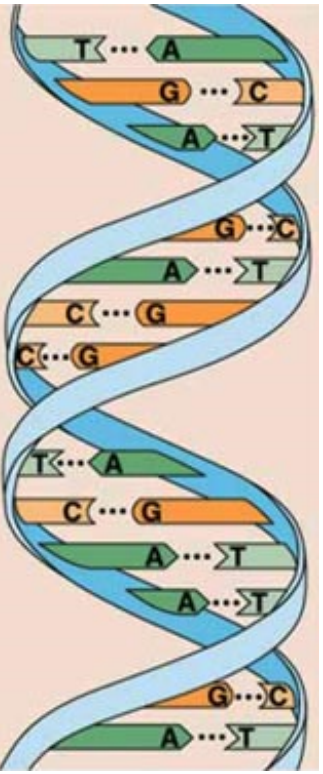
Trend towards abandonment of isolates

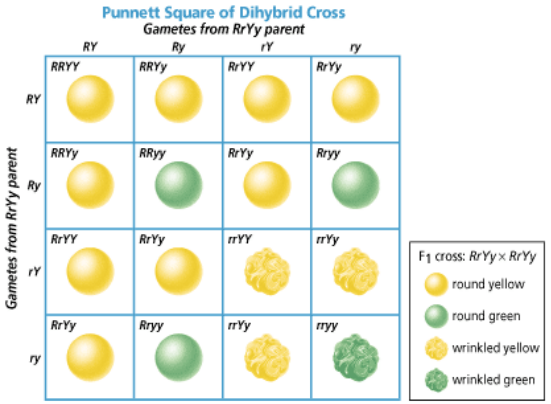


- CIDTs: Culture-independent diagnostic tests (NO ISOLATES)

- Reduce reliance on traditional (i.e. slower) culture-based
  - Nucleic acid-based:
    - Sensitive and specific
    - Detect toxin-producing genes or other biomarkers
    - E.g. PCR (singleplex, multiplex, quantitative, real-time), amplification such as LAMP, NASBA, DNA microarray, microfluidic chip, MALDI-TOF mass spectrophotometry.
  - Antigen-based:
    - ELISA
- When no isolates are recovered, Public Health Agencies lack evidence to link clinical cases to each other and to food.

Metagenomics





- More than just DNA, Mendel and the pea plants…
- Data mining of information obtained from metagenomics assays could provide the solution to a cultureless future in clinical microbiology, food safety and public health.
- Once optimized, can eliminate the need for culture.
- Will allow for rapid ID, gene content ID, virulence determination, AMR interpretation, etc.
- Generate immense quantities of large-scale sequence data, thus bioinformatics and other computational approaches are required to assign sequences, functions or other descriptors.

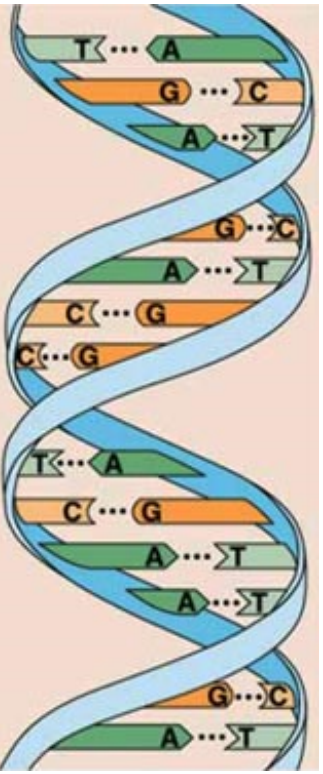
  (1) High-throughput targeted-amplicon metagenomics: target is specific to a particular microbial group (e.g. 16S rRNA: bacteria, 18S rRNA: eukaryotes)

  (2) Shotgun metagenomics (e.g. all DNA in a sample is sequenced)

Shotgun metagenomics

- 'Environmental DNA Sequencing' - Allows for analysis of all genes in all organisms present in a given complex sample, evaluate bacterial diversity and detect the abundance of microbes in various environments.
  - Relationships → microbial communities.
- Provides a means to study unculturable microorganisms that are otherwise difficult or impossible to analyze.
- Sequence thousands of organisms in parallel.
- Can detect very low abundance members of the microbial community that may be missed or are too expensive to identify using other methods.
- Usefulness in culture: for example, shotgun has shown enrichment media to detect *Salmonella* in tomatoes has allowed *Paenibacillus* spp. to out-complete and even kill *Salmonella,* suggesting alternative enrichment media should be used.

CIDT: example of current issues



- CDC reports 1973 – 2012 STEC was second only to noroviruses in number of leafy vegetable outbreaks, of which 46 of 49 were O157.
- STEC infectious dose: 10 – 100 CFU.
- BAM (FDA) culture method: enrich sample 24h, multiplex PCR for toxin genes.
- **Q**: How much contamination is necessary for detection without enriching the 'spinach' sample? + How long an enrichment is necessary for detection of very low STEC level?
- **A**: At 10 and 1000 CFU, STEC could not be detected.
  - At 100,000 cfu, detected without enrichment.
  - 5h enrichment not long enough to detect STEC at 10 CFU/100g spinach; 8h was shortest enrichment time for detection.
- Suggests overall microbial load in RTE spinach is too great for metagenomic approach without some enrichment.

Trend towards abandonment of isolates

Pros	Cons
Faster TAT: • Critical for clinical decision-making, decreases use of broad-spectrum ABs • Early outbreak detection and control • Food industry release or recall of products	• **No isolate** • Determination of viability • Application to predict phenotype is much less reliable (at present)
• More sensitive and specific than culture	• Sensitivity (?)
• ID new species, e.g. viruses etc.,	• Data is large and complex, complicating analysis
• Long term may be more cost effective than culture	• Currently relatively expensive
• Can ID non-culturable organisms & communities AND examine function	

Thank-you