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Whole Genome Sequencing—The Basics

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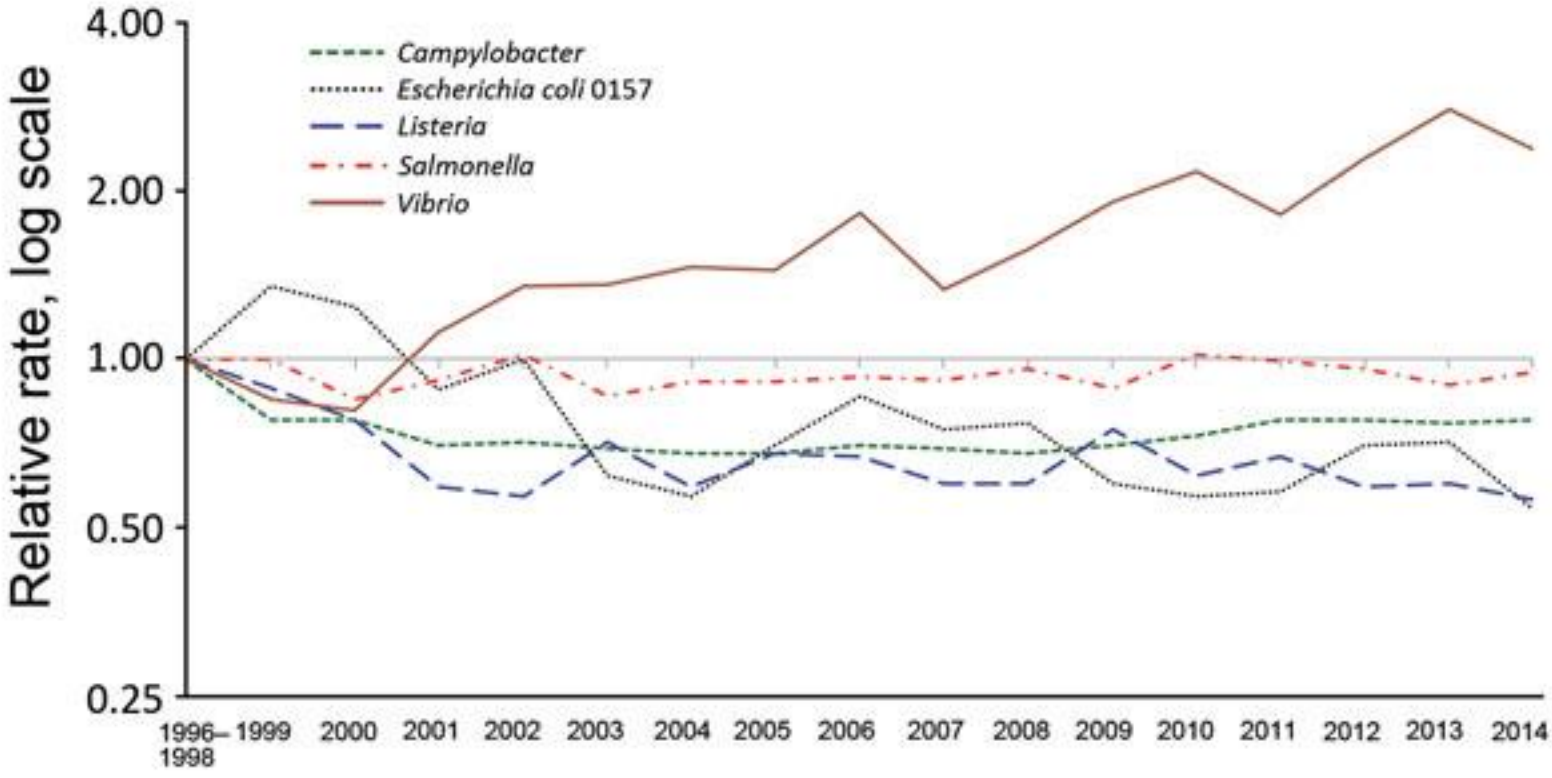
Estimated annual human health burden of selected known foodborne diseases, United States

Pathogen	Illnesses	Deaths	Case-fatality
Campylobacter	1,322,137	119	0.1%
Salmonella	1,229,007	452	0.5%
***E. coli* O157:H7**	96,534	31	0.5%
Listeria (LM)	1662	266	15.9%

CDC
Centers for Disease Control and Prevention

Scallan, et al., *Emerging Infectious Diseases, 2011*

Relative Rates of Foodborne Illness Compared to '96-98



http://wwwnc.cdc.gov/eid/article/21/9/15-0581-f2

2014 FOOD SAFETY PROGRESS REPORT

Pathogen	Healthy People 2020 target rate	2014 rate*	Change compared with 2006-2008[†]	
Campylobacter	8.5	13.45	13% increase	☹
E. coli O157[§]	0.6	0.92	32% decrease	☺
Listeria	0.2	0.24	No change	😐
Salmonella	11.4	15.45	No change	😐
Vibrio	0.2	0.45	52% increase	☹
Yersinia	0.3	0.28	22% decrease	☺

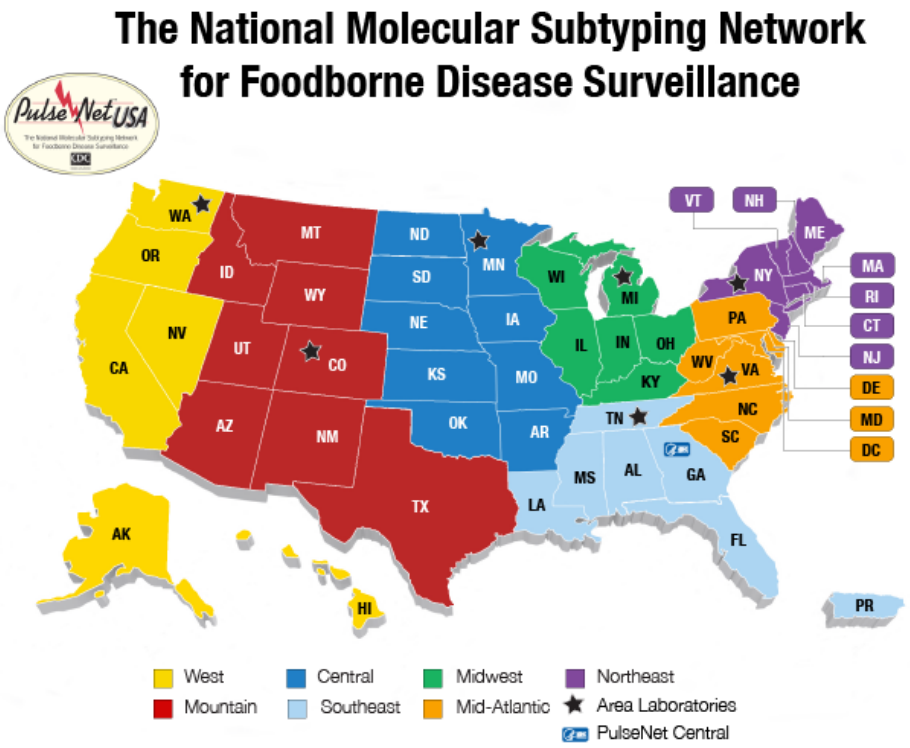
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

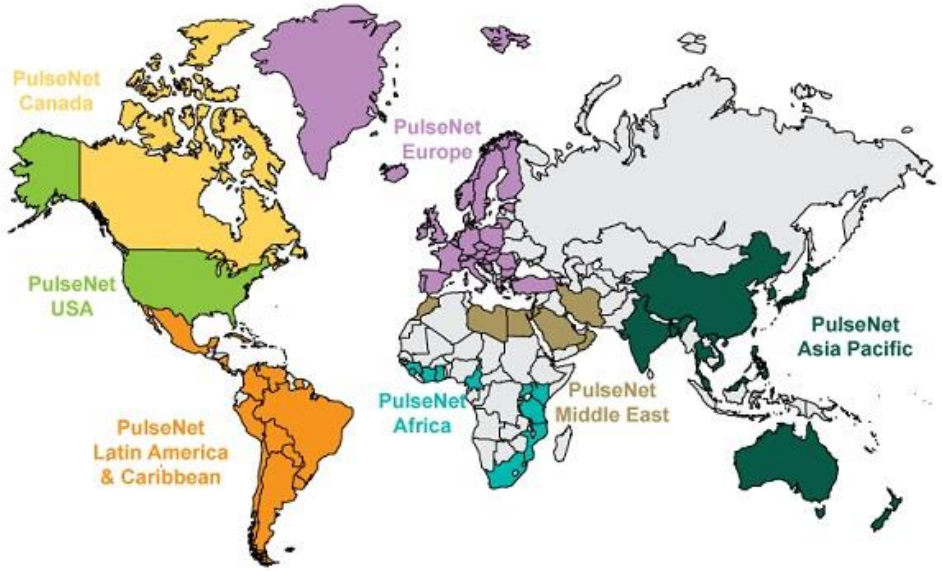
*Culture-confirmed infections per 100,000 population

[†] 2006-2008 were the baseline years used to establish Healthy People 2020 targets

[§] Shiga toxin-producing *Escherichia coli* O157

PulseNet USA & International





Subtyping & Outbreak Investigations

Exposure to Pathogen in Food

Human infection

Isolation from sterile body fluid using non-selective blood plates

Subtyping of Isolate

Food

Isolation using selective enrichment and selective and differential agar media

Subtyping of Isolates

Pulsed Field Gel Electrophoresis

The Workhorse from 1996-2015

The Pulsed-field Gel Electrophoresis Process

Bacterial Culture

1 The scientist takes bacterial cells from an agar plate.

Mix bacteria with Agarose

2 The scientist mixes bacterial cells with melted agarose and pours into a plug mold.

Plug Mold

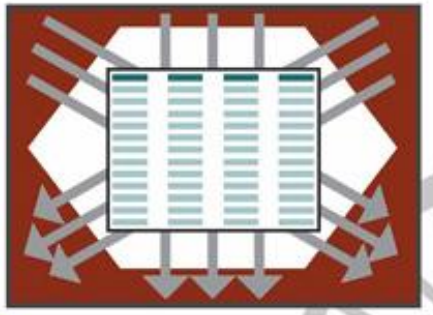
Lyse Cells and Wash Plugs

3 The bacterial cells are broken open with biochemicals, or lysed, so that the DNA is free in the agarose plugs.

DNA is now in Plugs

Cut DNA with Restriction Enzyme

Pulsed-field Gel Electrophoresis (PFGE)



4 The scientist loads the DNA gelatin plug into a gel, and places it in an electric field that separates DNA fragments according to their size.

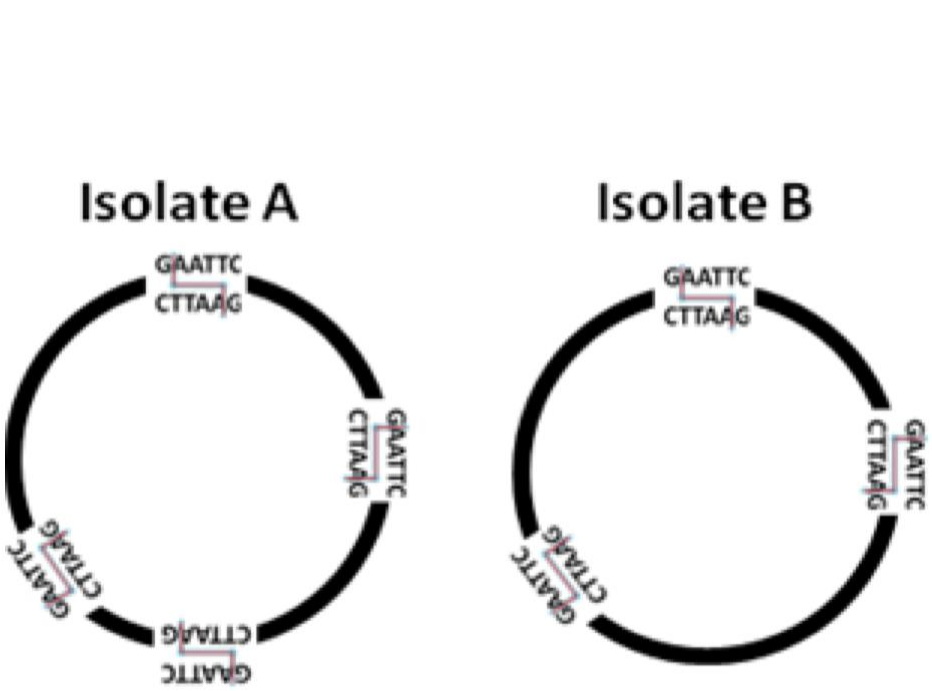
Data Analysis (BioNumerics)

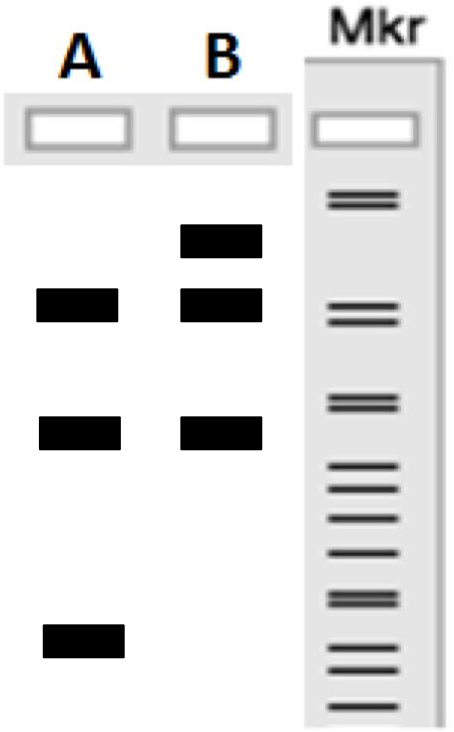
5 The gel is stained so that DNA can be seen under ultraviolet (UV) light. A digital camera takes a photograph of the gel and stores the picture in the computer.

CS238193-A

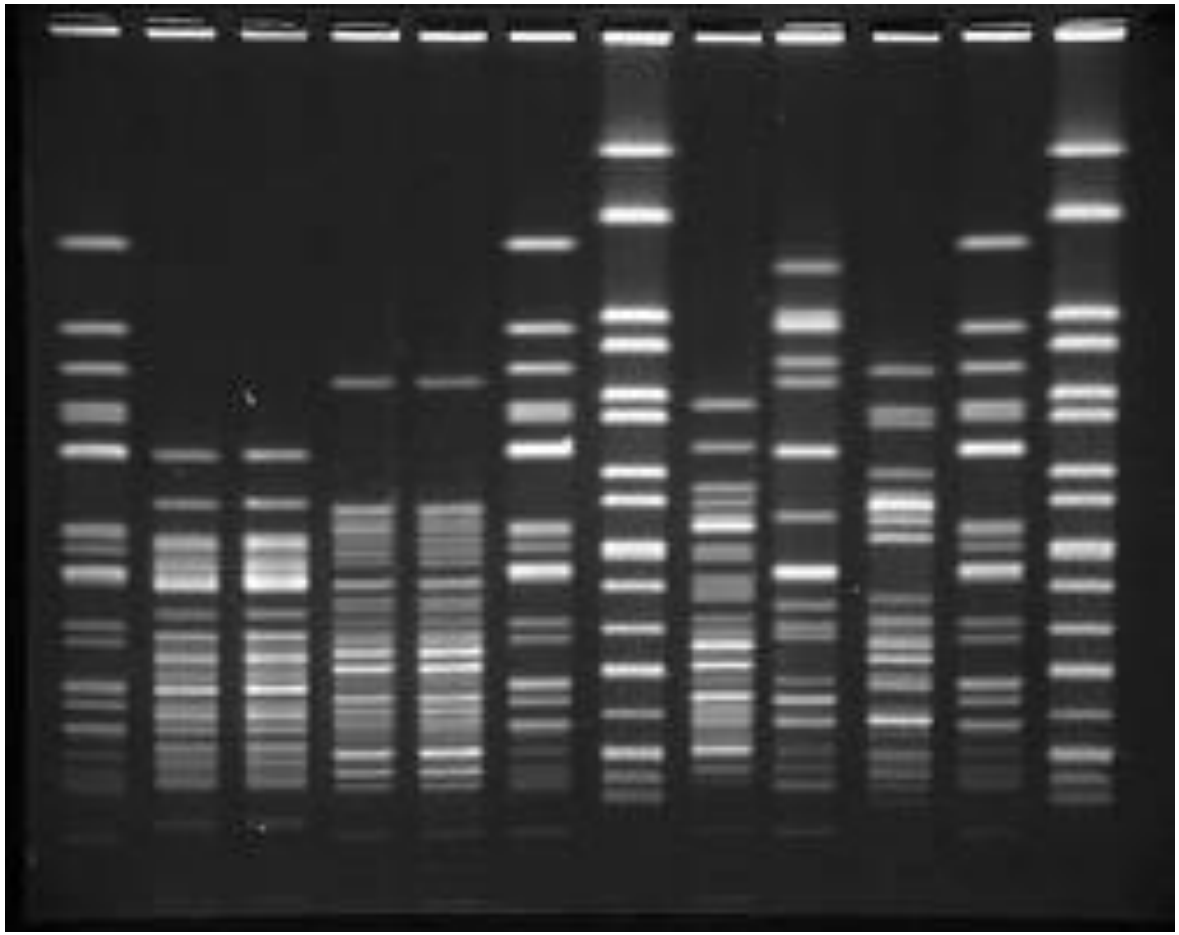
http://www.cdc.gov/pulsenet/pathogens/protocol-images.html#pfge

Restriction Enzymes = Molecular Scissors





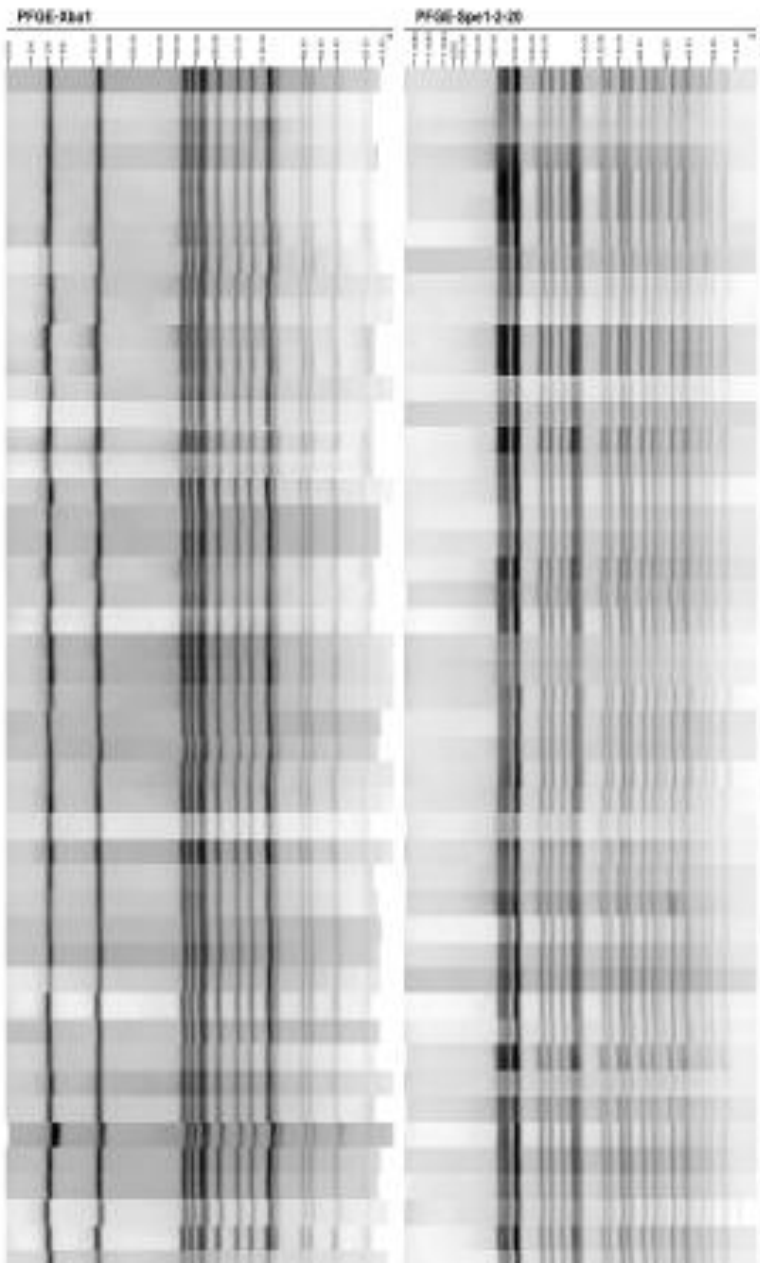
PFGE Gel



Challenges of PFGE in Outbreak Investigations

- Two isolates may have same PFGE type even though genetically distinct

- Two isolates may show “slightly” different PFGE patterns despite sharing a very recent common ancestor



- Salmonellosis outbreak linked to sausages (Rhode Island) and isolates from pistachios
- JIXX01.0011 is the most common among 1,225 S. Montevideo isolates in the PulseNet database

Den Bakker et al., 2011
doi: 10.1128/AEM.06538-11

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	April	May	June	July	August	September	October	November	December
Food Contact Sites									
Slicer	-	-	-	-	-	-	-	-	-
Deli case	NT	NT	NT	-	-	-	-	-	-
Deli case near raw meat	NT	NT	NT	-	-	-	-	-	-
Deli case trays	NT	NT	NT	-	-	-	-	-	-
3-basin sink interior	NT	NT	NT	CU-57,267	-	-	-	-	-
1-basin sink interior	NT	NT	NT	CU-258,69	-	-	-	CU-294,321	-
Cold room rack	-	-	-	-	-	-	-	-	-
Cutting board	NT	NT	NT	NT	-	-	NT	NT	-
Rewrap table	NT	NT	NT	-	-	-	-	-	-
Counter	NT	NT	NT	-	-	-	-	-	-
Non-food contact sites									
3-basin sink exterior	NT	NT	NT	-	-	-	-	-	-
Floor/wall junction (3-basin)	CU-258,69	CU-258,69	CU-258,69	CU-258,69	CU-258,69	CU-8,96	LM	CU-258,69	CU-258,69
1-basin sink exterior	NT	NT	NT	CU-258,69	-	-	LM	-	CU-258,69
Floor/wall junction (1-basin)	NT	NT	NT	CU-258,69	-	-	LM	CU-258,69	CU-258,69
Deli drain	NT	NT	NT	CU-258,69	CU-258,333	-	CU-258,69	CU-258,69	CU-258,69
Floor adjacent to drain	-	CU-258,69	CU-258,69	CU-258,69	CU-258,69	-	-	CU-258,69	CU-258,69
Deli floor	NT	NT	NT	CU-258,69	-	-	-	CU-258,69	-
Cold room floor	NT	NT	NT	CU-258,69	CU-295,329	-	CU-258,69	CU-258,69	CU-258,69
Cold room wall	CU-258,69	-	-	-	-	-	-	-	-
Cold room drain	NT	NT	NT	CU-258,69	CU-258,69	-	CU-258,69	CU-258,69	CU-258,69
Standing water	NT	NT	NT	NT	-	-	NT	NT	-
Squeegee	NT	NT	NT	CU-258,69	CU-258,69	-	CU-258,69	CU-258,69	CU-258,69
Cart Wheel	-	-	CU-258,69	CU-258,69	-	-	-	-	-
Hose	NT	NT	NT	-	CU-258,69	-	-	-	-
Trash can	-	-	CU-258,69	-	CU-258,69	-	-	-	-
Transfer Points									
Slicer knob	-	-	-	-	-	-	-	-	-
Case handle	-	-	-	-	-	-	-	-	-
Scale	NT	NT	NT	-	-	-	-	-	-

	August	September	October	November	December	January
Food Contact Sites						
[illegible]	-	-	-	-	-	-
[illegible]i case	-	-	-	-	-	-
Deli case near raw meat	-	CU-259,322	-	-	-	-
Deli case trays	-	-	-	-	-	-
3-basin sink interior	-	-	-	-	-	-
1-basin sink interior	CU-40,96	CU-296,330	CU-57,267	CU-296,330	-	LM
Cold room rack	-	-	-	-	-	-
Cutting board	-	CU-262,79	-	-	-	-
Rewrap table	-	-	-	-	-	-
Counter	-	-	-	-	-	-
Non-food contact sites						
3-basin sink exterior	-	-	-	-	-	-
Floor/wall junction (3-basin)	CU-258,323	CU-258,322	-	-	-	LM
1-basin sink exterior	-	-	-	-	-	-
Floor/wall junction (1-basin)	CU-258,323	CU-258,323	-	CU-258,69	-	LM
Deli drain	CU-259,322	CU-258,323	CU-11,320	CU-262,334	-	LM
Floor adjacent to drain	CU-259,322	CU-262,317	CU-258,322	LM	CU-258,322	LM
Deli floor	CU-258,333	CU-258,323	-	-	-	LM
Cold room floor	CU-258,322	-	CU-258,322	-	-	LM
Cold room wall	-	-	-	-	-	-
Cold room drain	CU-258,322	CU-259,322	CU-258,67	LM	CU-258,323	LM
Standing water	CU-82,215	NT	CU-298, 335	NT	CU-258,323	-
Squeegee	CU-259,322	CU-258,322	CU-262,334	LM	CU-259,322	LM
Cart Wheel	-	-	CU-258,323	-	-	-
Hose	-	-	-	-	-	-
Trash can	-	CU-258,322	-	-	-	LM
Transfer Points						
Slicer knob	-	-	-	-	-	-
Case handle	-	CU-258,322	-	-	-	-
Scale	-	-	-	-	-	-

Transitioning from PFGE to WGS

- Fast
- Reduced cost
- Increased discriminatory power

"Whole-genome sequencing shines a new light in this area that helps us find unsuspected gaps in the food safety system… this is a wake-up call to the ice cream industry in general and to the regulators."

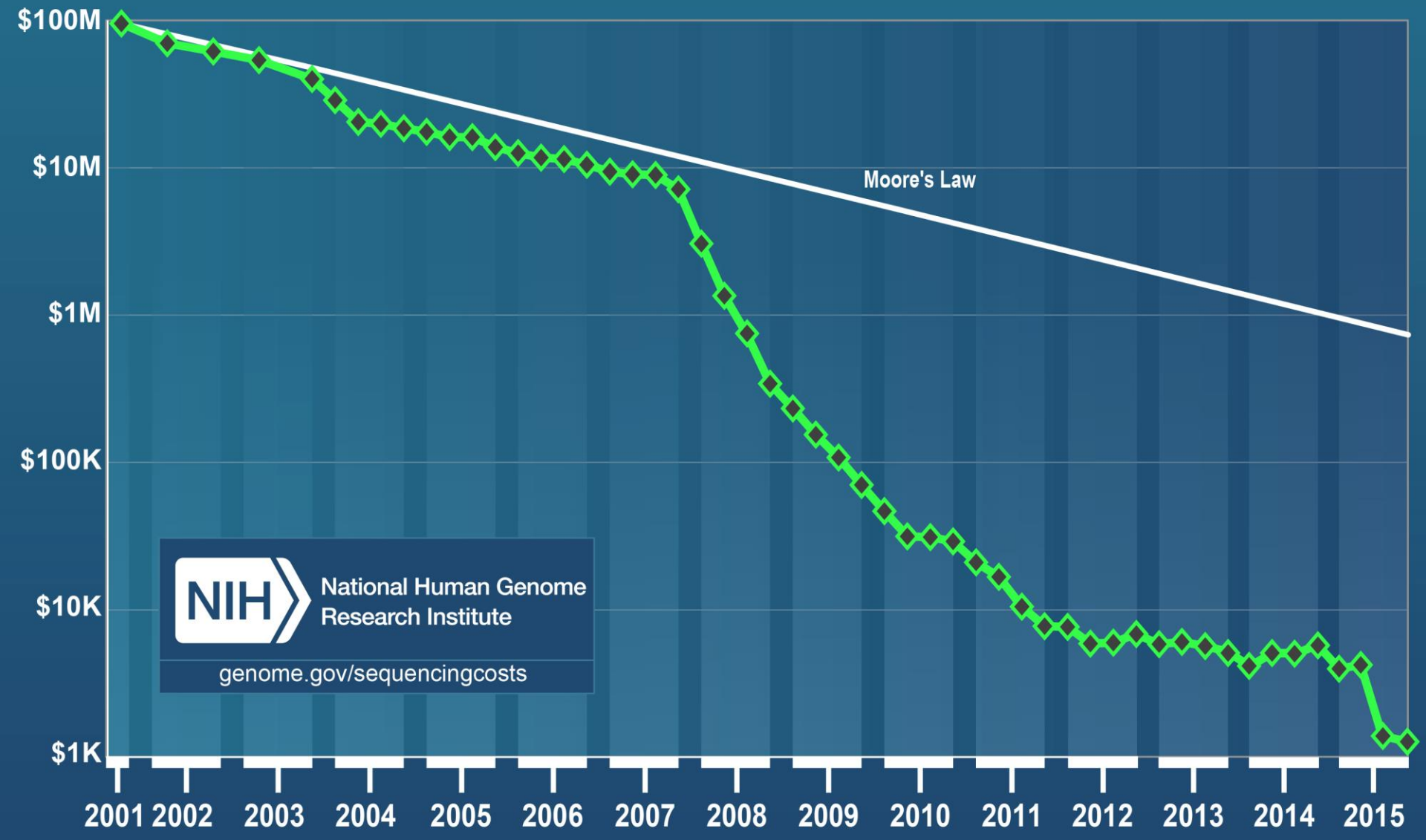
~Robert Tauxe, CDC

DOI: 10.1126/science.aac4551

Why WGS?

- Fast
- Reduced cost
- Increased discriminatory power

Cost per Genome



History of Sequencing

- Sanger (radioisotopes)
- Sanger (fluorescent dye chemistry)
- SOLiD
- 454
- Illumina
- Ion Torrent
- PacBio
- MinION

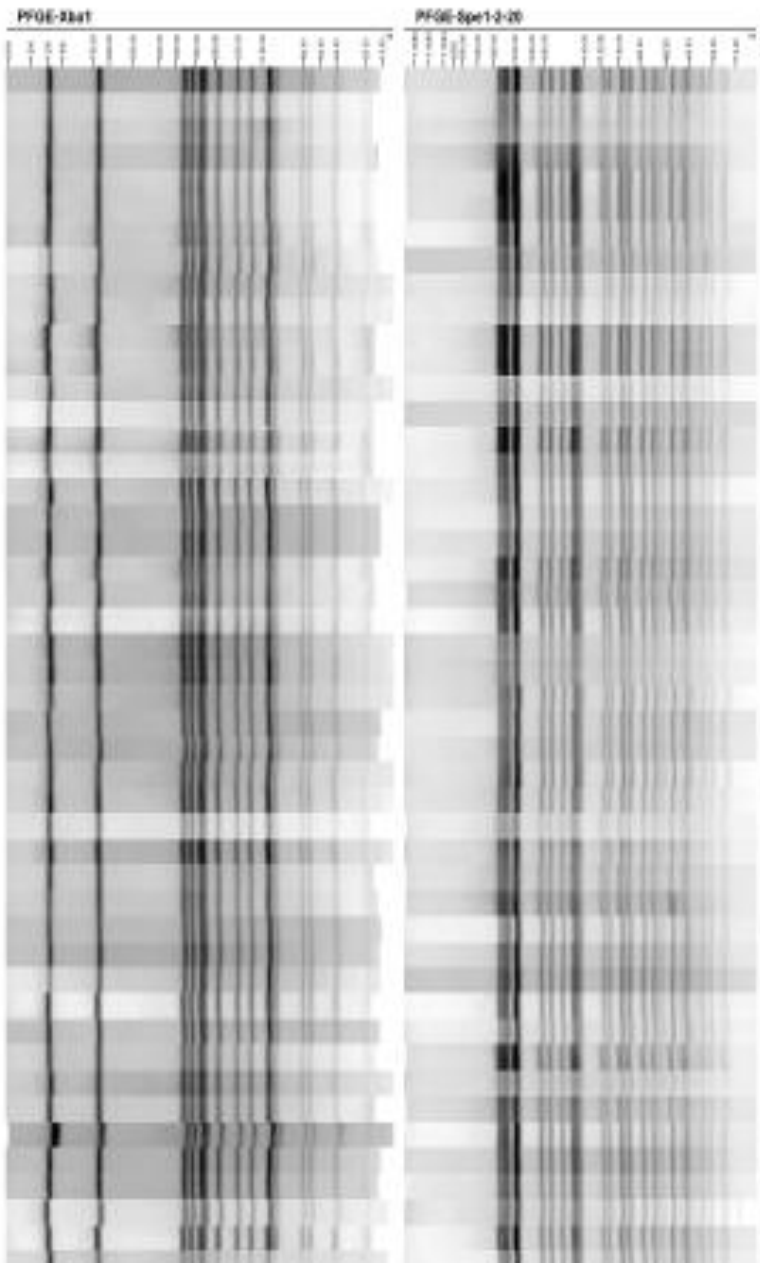
PCR = Basis of these technologies

Basics of Cycle Sequencing

Cycle sequencing.html

Illumina HiSeq and MiSeq Sequencing by Synthesis

Illumina Youtube



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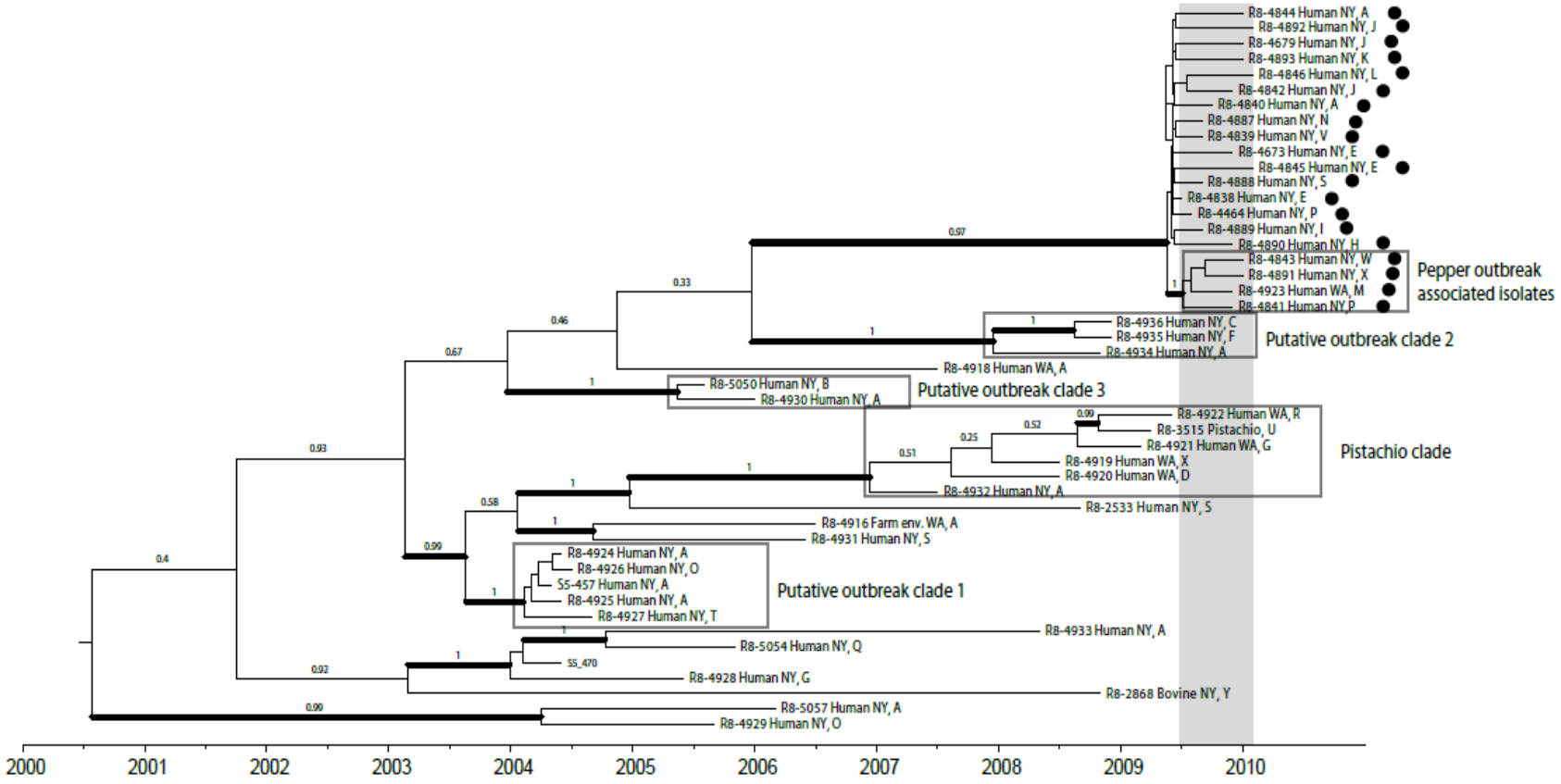
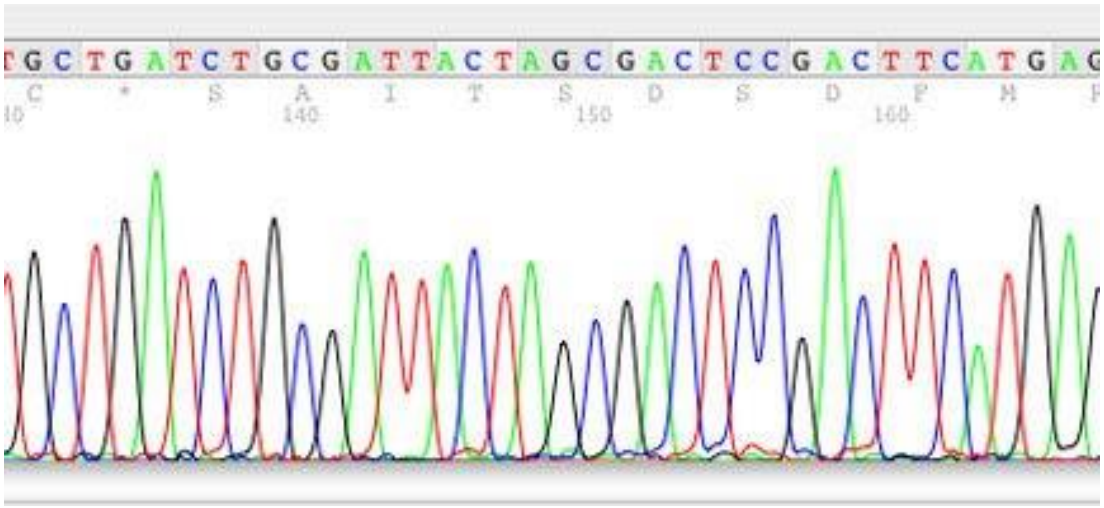
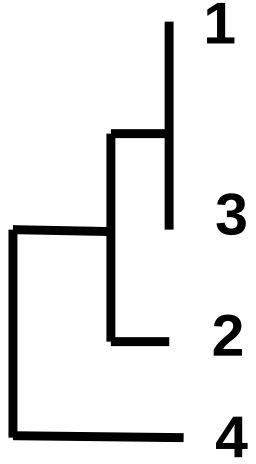
Tip-dated maximum clade credibility tree based on SNP data for 47 Montevideo isolates



DNA sequencing-based subtyping





Isolate 1	AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 2	AACATGCAGACTGACGATTCGTCGTAGGCTAGACGTTGACTG
Isolate 3	AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 4	AACATGCATACTGACGATTCGTCGAAGGCTAGACGTTGACTG

SNP: single nucleotide polymorphism