IDFA
International
Dairy Foods Association

Leveraging New Molecular Technologies to Improve Dairy Operations

MERIEUX NutriSciences

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Welcome & Introductions

Joseph Scimeca - Senior Vice President, Regulatory & Scientific Affairs, IDFA

HOUSEKEEPING

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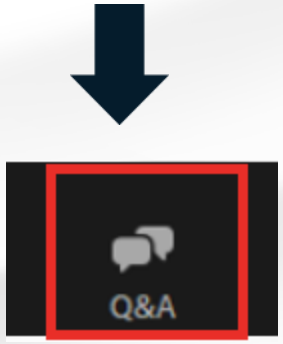
- All lines are placed on mute during this webinar.
- The recording and slides will be available in the Knowledge Center, under the Webinars tab.
- For technical difficulties please send an e-mail to membership@idfa.org.
- Please submit questions through the Q&A feature throughout the webinar and we will answer them at the conclusion of the webinar.



- **Step 1:** Select the Q&A bubble
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CO-MODERATOR

John Allan - Vice President, Regulatory Affairs and International Standards, IDFA

PRESENTERS

Tim Freier
VP of Microbiology & Scientific Affairs, Merieux NutriSciences

Sarita Raengpradub Wheeler
Director Microbiology R&D, Merieux NutriSciences

Leveraging New Molecular Technologies to Improve Dairy Operations

Sarita Raengpradub Wheeler & Timothy Freier

August 12, 2020

MERIEUX NutriSciences

Old Problems

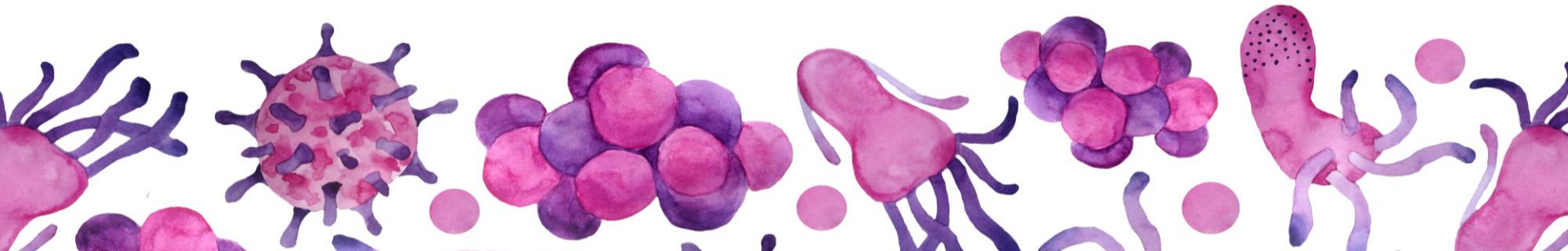
Ongoing micro concerns

FSMA Environmental Pathogens

Spoilage

Determining shelf life of new or reformulated products

- *Listeria monocytogenes* in cheese, butter, ice cream
- *Salmonella* and *Cronobacter* in spray-dried dairy products
- Persistent vs. transient



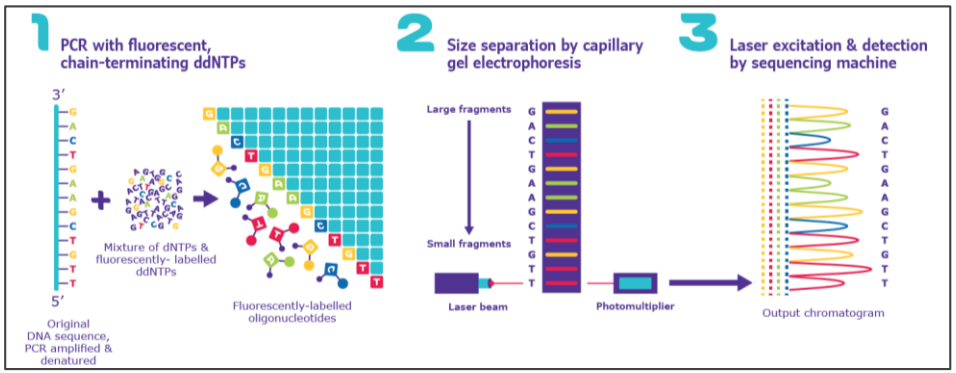
Old Problems

- Unusual or unanticipated issues
  - **1994 ice cream, *S.* Enteritidis – 224,000 illnesses**
    - Premix contaminated by tankers that hauled raw eggs
    - Before WGS, but 3 phage types found from case patients
    - Found both *S.* Enteritidis and *S.* Thompson in ice cream samples
    - Highest level found was 6 cells/half-cup serving, rest of samples were <0.2 cells/serving
  - **2013 whey protein concentrate, *Clostridium botulinum* – massive recalls of infant formula, protein drinks and sports drinks**
    - China completely banned imports of milk powder products from New Zealand
    - Was eventually determined that it was a misidentified *C. sporogenes*
  - **2013 Greek yogurt, mold – over 400 illnesses**
    - Bloating, nausea, diarrhea and vomiting
    - Mold ID'ed as *Mucor circinelloide*s, thought that symptoms might be psychosomatic, not a gastrointestinal pathogen
    - 10 months later – study at Duke University – WGS indicated it may have been a subspecies more associated with illness, and potentially could have produced harmful metabolites previously unknown in that species

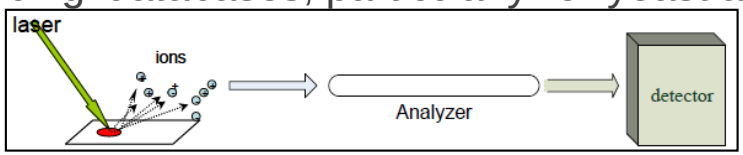
NEW SOLUTIONS

Identification

- Old: Biochemical methods – days to weeks for results
- Newer: Can be more definitive
  - Sanger sequencing – using ribosomal RNA genes for ID
    - Gold standard, single genes



  - MALDI-TOF MS: Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry – using ribosomal proteins for ID
    - Expanding databases, particularly for yeast and mold

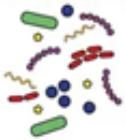


- Newest: Next Generation Sequencing - more information at one time
  - WGS: Whole Genome Sequencing
  - Metagenomics or metabarcoding

Image Credit: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/articles/biology/marketing-assets/sanger-sequencing_steps_process_diagram.png

Identification & Characterization: By Molecular Technologies

Mixed microbial communities

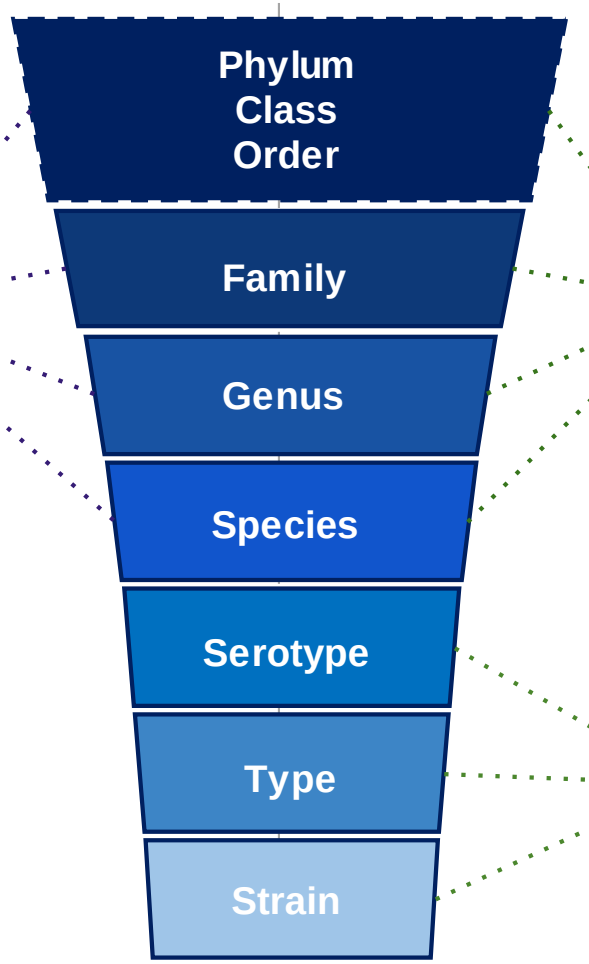


Taxonomic Levels

Isolated microorganisms

Identification & Characterization

Next Generation Sequencing
- Metagenomics
- Metabarcoding



Identification

Sanger Sequencing

MALDI-TOF MS

Characterization

Molecular Serotyping

Molecular Subtyping
(RiboPrinter, PFGE, MLST)

WGS
(wgMLST, wgSNP)

Next Generation Sequencing

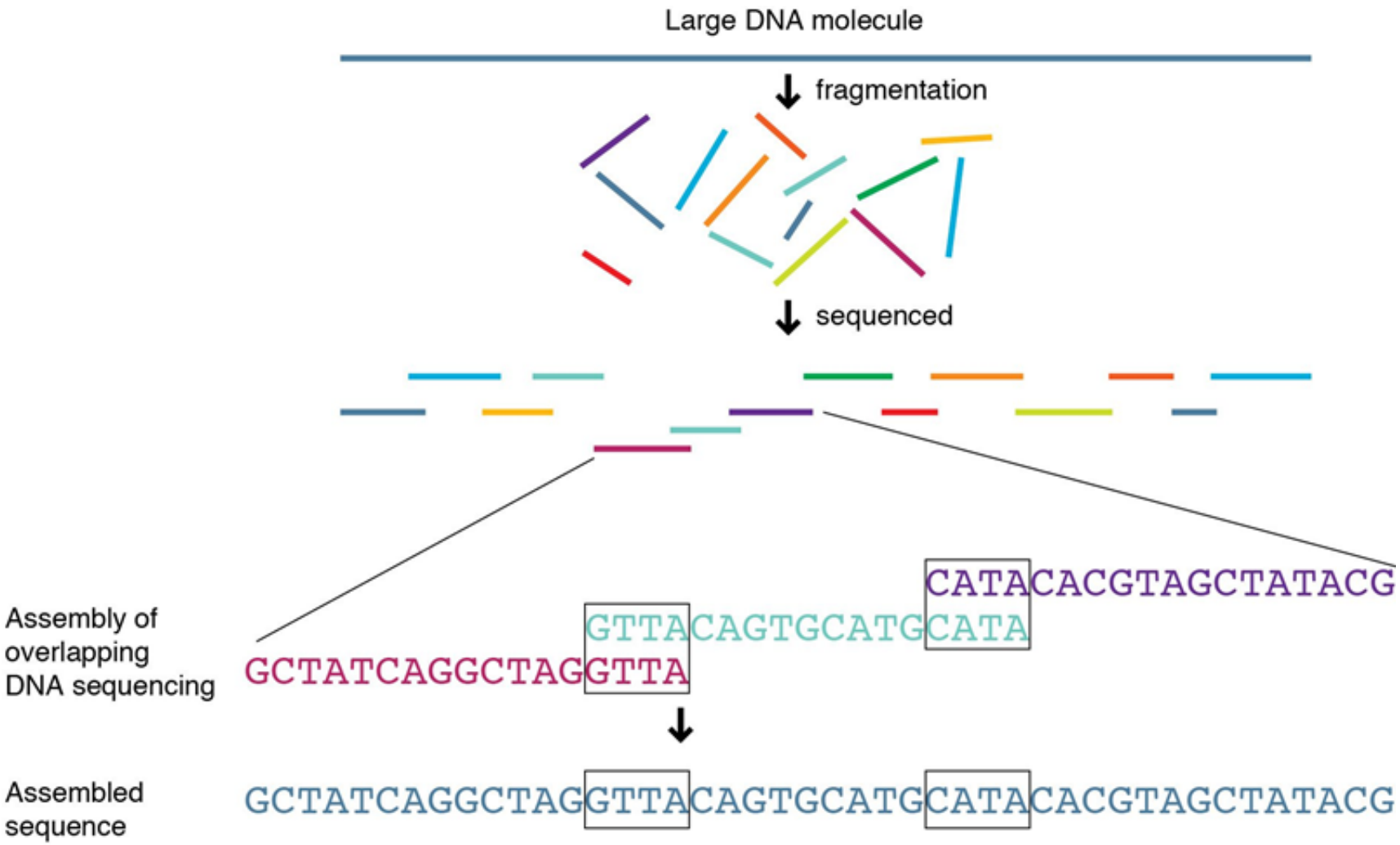


Image credits
http://knowgenetics.org/wp-content/uploads/2012/12/kevin2-1024x623.png

Technology Evolves

First Generation	Second Generation	Third Generation
Short or long read sequencing	Short read sequencing	Long read sequencing
Sequencing time = hours	Sequencing time = days	Sequencing time = can be hours
Analysis = follows sequencing	Analysis = follows sequencing	Analysis = can be real-time (shorten analysis time)
Not computationally intensive	Computationally intensive	Still computationally intensive!

Image credits:
Thermo Fisher Scientific, Illumina, Oxford Nanopore Technologies, Pac Bio

What is the NGS question?

Next Generation Sequencing

Metagenomics (Shotgun)

Targeted Metagenomics (Metabarcoding)

Whole Genome Sequencing

"Who is here?"

"In what proportion?"

"What can they do?"

"Which strain is this?"

"What makes this strain unique?"

How does NGS work?

Sample Preparation

- *Extract DNA*
- *Prepare sequencing library*

Sequencing

- *Generate millions of DNA pieces*

Bioinformatics

- *Assemble all DNA pieces*
- *Compare against a reference (e.g., genome, typing scheme) or database*

Whole Genome Sequencing

Whole

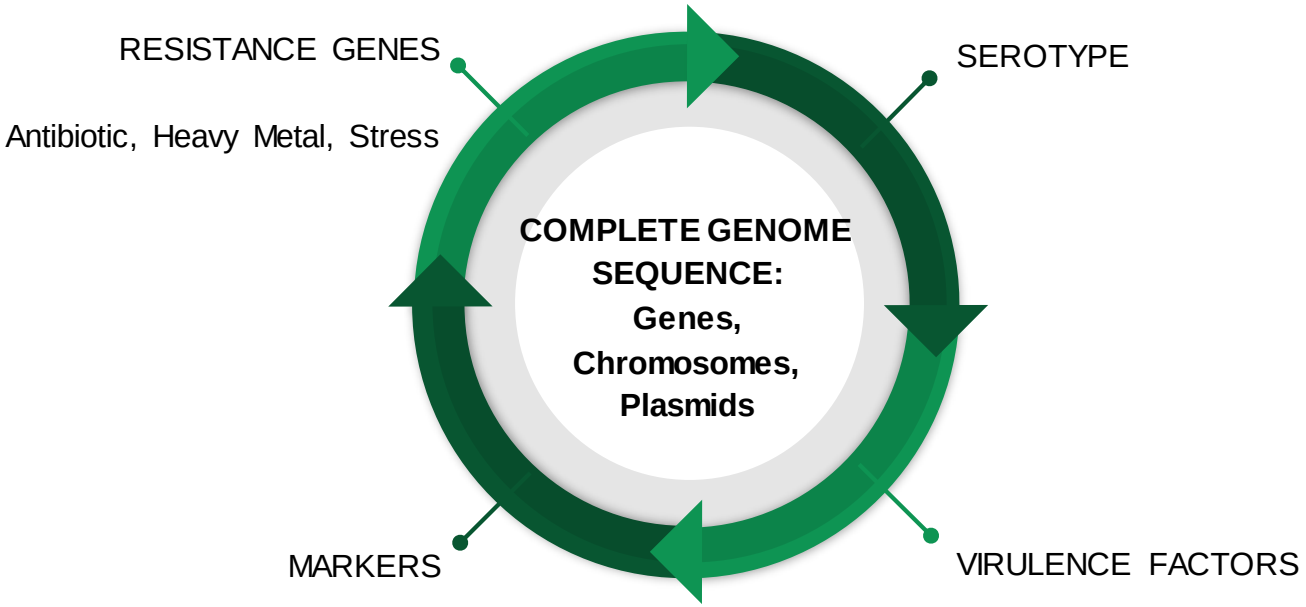
"Genome" = "gene" + "chromosome"

Genome

The genome sequence is made up of a series of letters (A, C, G, T) organized in base pairs

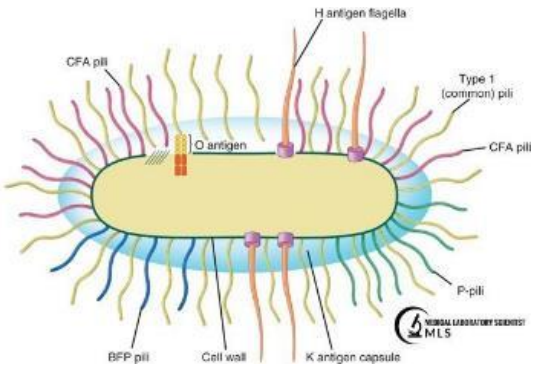
Sequencing

WGS analysis looks at nucleotide level to determine what is different between strains of the same species



Typing Methods

Classical Solutions



New Solutions

Image credits: Hygiena, Bio-Rad, Rheonix, Illumina, Oxford Nanopore Technologies

Molecular Serotyping

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FOOD MICROBIOLOGY

Performance and Accuracy of Four Open-Source Tools for *In Silico* Serotyping of *Salmonella* spp. Based on Whole-Genome Short-Read Sequencing Data

Laura Uelze,[a] Maria Borowiak,[a] Carlus Deneke,[a] István Szabó,[a] Jennie Fischer,[a] Simon H. Tausch,[a] Burkhard Malorny[a]

[a]German Federal Institute for Risk Assessment (BfR), Berlin, Germany

SCIENTIFIC REPORTS
nature research

Genome-based *Salmonella* serotyping as the new gold standard

Sangeeta Banerji[1,3], Sandra Simon[1,3], Andreas Tille[2], Angelika Fruth[1] & Antje Flieger[1*]

Salmonella enterica is the second most reported bacterial cause of food-borne infections in Europe. Therefore molecular surveillance activities based on pathogen subtyping are an important measure of controlling Salmonellosis by public health agencies. In Germany, at the federal level, this work is carried out by the National Reference Center for *Salmonella* and other Bacterial Enteric Pathogens (NRC). With rise of next generation sequencing techniques, the NRC has introduced whole-genome-based typing

ORIGINAL RESEARCH
published: 03 April 2020
doi: 10.3389/fmicb.2020.00549

Systematic Evaluation of Whole Genome Sequence-Based Predictions of *Salmonella* Serotype and Antimicrobial Resistance

*Ashley L. Cooper[1,2], Andrew J. Low[1], Adam G. Koziol[1], Matthew C. Thomas[3], Daniel Leclair[4], Sandeep Tamber[5], Alex Wong[2], Burton W. Blais[1,2] and Catherine D. Carrillo[1]**

ELSEVIER

Food Microbiology
Volume 89, August 2020, 103452

Evaluation of real-time nanopore sequencing for *Salmonella* serotype prediction

Feng Xu [a], Chongtao Ge [a], Hao Luo [a], Shaoting Li [b], Martin Wiedmann [c], Xiangyu Deng [b], Guangtao Zhang [a], Abigail Stevenson [a], Robert C. Baker [a], Silin Tang [a]

Molecular Serotyping

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- Pure culture to serotyping in the same day
- Two NGS technologies (Illumina, Oxford Nanopore Technologies) combined with web-based serotyping tools (SeqSero, SISTR)

Illumina

Benchtop sequencing

WGS by Illumina
- **library prep (1-2 days)**
- **sequencing (2 days)**
- **bioinformatics (2+ days)**

Oxford Nanopore

Portable, real-time sequencing

WGS by ONT
- **library prep (<3h)**
- **sequencing (<2h)**
- **bioinformatics (<1 day)**

CRISPR Typing

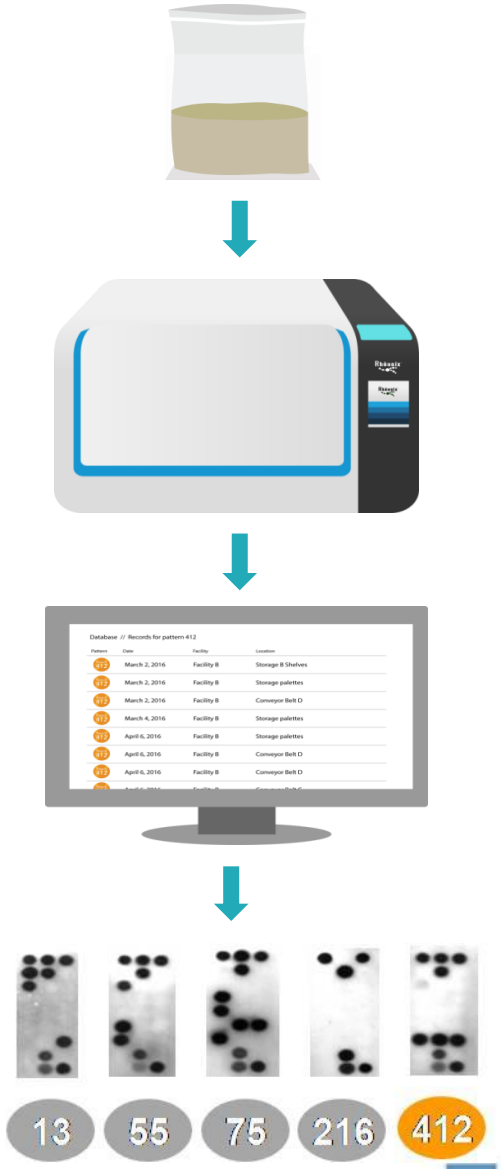
- CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats
- *Salmonella* have two CRISPR loci that can be considered serovar specific
- CRISPR-SeroSeq: Serotyping by sequencing of the CRISPR loci
- Utilized NGS

Typing by Rheonix Technology

Rheonix®

***Listeria* Subtyping Platform**

- Ability to **detect multiple strains** in an enrichment
- Uses multiple target probes on a low-density microarray; **automated platform**
- Each pattern is a **molecular signature** that can be compared user-specific database
- **Creates patterns** by testing for the presence or absence of genomic sequences present in *Listeria* strains



Time to Results Comparison

Current methods of subtyping take 1-2 weeks

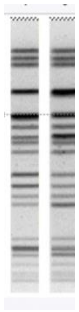
Sample Enrichment (1-2 days)

Pathogen Screen (1-4 hours)

Culture Isolation (3-4 days)

Strain Typing by PFGE or WGS (1-7 days)

~ 9 days



Sequence
TTACAACGGGAGAAGACAATGCCACCA
ATGTCATGGAATAAT
TGCACTGGTTTAGCTTGGGAATGGT
TTTGCAAGTCAGGGTCTTGTCCGA
AGGCGTATACACTCAGGAGAAGATAAAGGT
AGTATGACTTCGGGCACAGTTGGC
TGGCAGTTTCCAGGACTTCACTTGT
GGCGCGCCTAACACATCT

RESULT

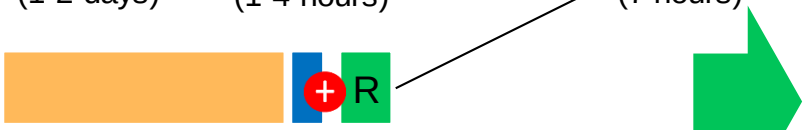
Detailed strain type info including pathogenicity and sequence / PFGE data that can be matched to outbreak strain

Rheonix Method – 2-3 days*

Sample Enrichment (1-2 days)

Pathogen Screen (1-4 hours)

Rheonix Typing: Direct from Enrichment (7 hours)



~ 2 days

	Pattern New	Seen Before
S1	X	
S1	X	
S3		X

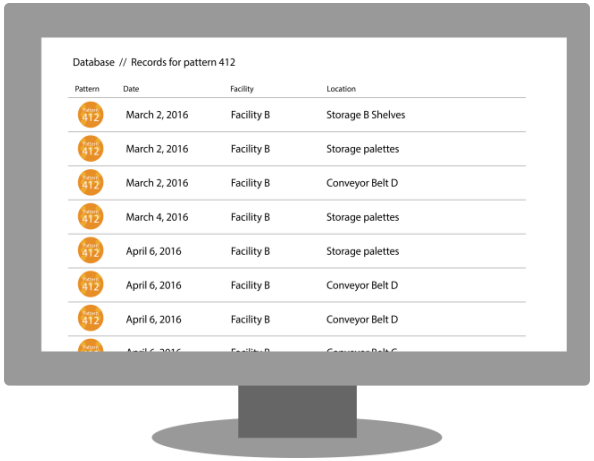
RESULT

Information about persistence of strain in facility – not comparable to public strain data

Slide courtesy of Rheonix

Listeria PatternAlert™ Assay

- Two assay formats
- Validated for environmental enrichments
- Works directly from presumptive enrichments but can also work with isolates
- Customer-specific database
  - Pattern 1 in ABC database (i.e., ABC-1) could be different than Pattern 1 in XYZ database (i.e., XYZ-1)
- Patterns do <u>not</u> show genetic relatedness
  - Pattern can reflect strains from multiple species

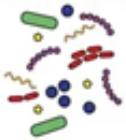


Example:
ABC-1 contains LS1 & LS 2.
ABC-1 can be found in *L. seeligeri* & *L. innocua*.

Have I seen ABC-1 before?

Identification & Characterization: By Molecular Technologies

Mixed microbial communities

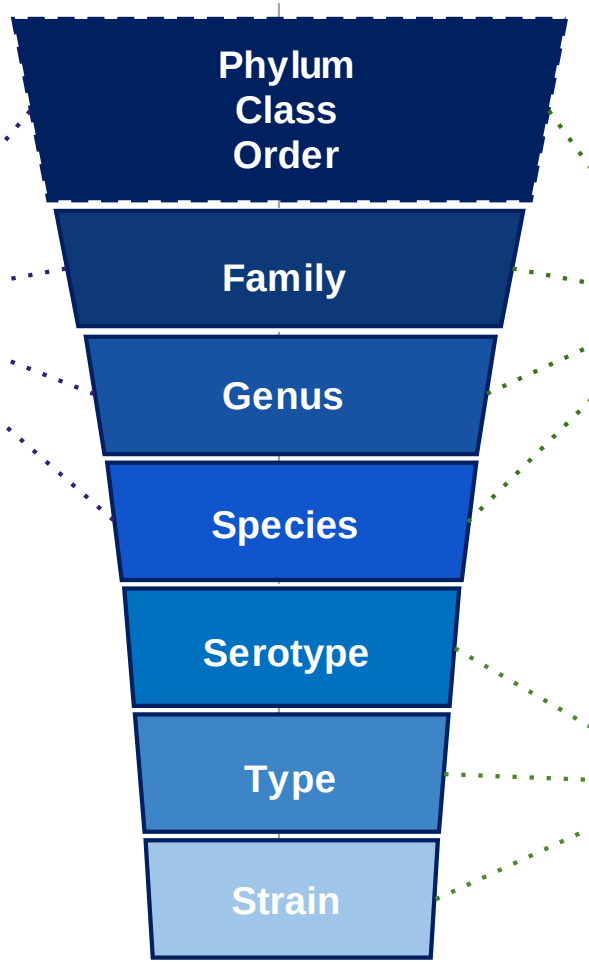


Taxonomic Levels

Isolated microorganisms

Identification & Characterization

Next Generation Sequencing
- Metagenomics
- Metabarcoding



Identification

Sanger Sequencing

MALDI-TOF MS

Characterization

Molecular Serotyping

Molecular Subtyping (Rheonix)

WGS (wgMLST, wgSNP)

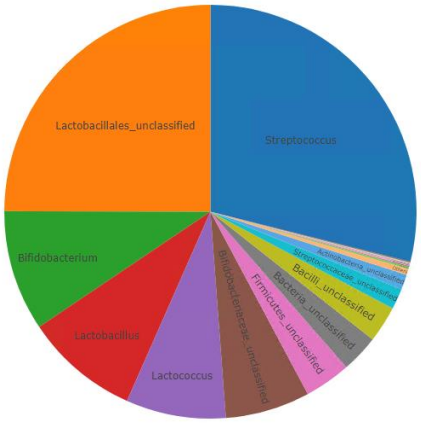
Metagenomics

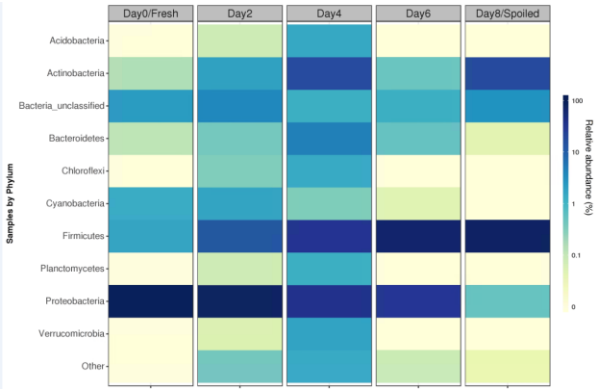
Metagenomics = Study of the metagenome

Metagenome = Collective genome of all organisms found in a given environment

Direct sequencing Study microbes without the need for isolation and cultivation of individual strains

Microbial profiling Surveying the microbial community within a sample (may not be to species leel)





Shotgun Metagenomics

- Sequence all DNA
- Host DNA contamination

Metabarcoding

- Amplify specific DNA then sequence
- Reduces sequencing & data analysis burdens

Applying New Solutions to Old Problems

Environmental pathogens

- Rapid subtyping – our goal is to bring it out of the research lab and into routine testing
- Make mapping of strains routine (EnviroMap)
- Develop a facility-specific database to understand where the “house bugs” live, speeding investigation and corrective actions
- Differentiate persistent from transient for a better understanding of risk and keep a step ahead of the regulators
- Potential future benefit – illuminate strains of the same pathogen in an enrichment

Example

- There is a persistent strain of *Listeria*
- Mapping indicates there is no obvious harborage site in facility, but appears often in raw ingredient area
- The strain is found in a supplier’s ingredient – it is a persistent strain in their facility, and actually a transient strain in yours!

Applying New Solutions to Old Problems

Spoilage

- Molecular techniques such as Sanger Sequencing and Maldi-Tof can better identify predominant strains in spoiled product
- Many spoilage issues are complex – can be a sequence of organisms that trigger, not just the last one that predominates

Example

- Sample at multiple time-points
- Use metabarcoding to look at predominant taxa at each time point
- You find that spoilage only occurs when a certain group of bacteria predominate during the first week of shelf life, then a second type grows up and produces gas and slime by week three
- You determine through further NGS work that the first group of bacteria metabolizes the food matrix to produce a substrate that stimulates the growth of the second group

Applying New Solutions to Old Problems

Shelf Life

- Challenge studies
  - Instead of inoculating your product with a single or limited set of spoilage organisms, look at the total naturally-existing flora with metagenomics
- See how changes in formulation (antimicrobials, pH, Aw, packaging atmosphere, etc.) impact the total microflora with metagenomics

Applying New Solutions to Unusual or Unanticipated Events

- *Salmonella* in ice cream
  - Epidemiology would definitely be faster/better today with WGS
  - Routine rapid serotyping as strain tracking via NGS combined with robust EMP may have detected earlier
  - Ability to ID multiple strains of *Salmonella* in a product enrichment (Enteritidis and Thompson, maybe others) may have pointed finger to raw egg earlier
- *C. botulinum* in whey protein concentrate
  - Better molecular ID methods could have avoided $Millions in recall expenses
- Mold in yogurt
  - Molecular techniques were used, but long after the fact
  - More routine use of NGS, looking for genes for metabolites rather than trying to apply a genus and species name may indicate risk sooner

Interactive Polling Question

- Of the new technologies discussed, which one do you think holds the most promise for improving your operations?
  A. Improved microbial identification (Sanger sequencing or MALDI-TOF MS)
  B. Whole Genome Sequencing
  C. *Salmonella* molecular serotyping
  D. *Listeria* strain tracking (Rheonix)
  E. Metagenomics

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QUESTIONS?

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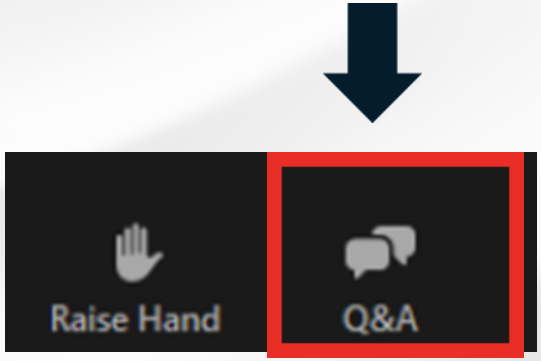

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THANK YOU!