TECHNOLOGY IN THE FOOD SAFETY WORLD: TOOLS SUCH AS WHO GENOME SEQUENCING – FRIEND OR FOE?

Room 314 | December 5 2017
CEUs – New Process

Certified Crop Advisor (CCA)
- Sign in and out of each session you attend.
- Pickup verification sheet at conclusion of each session.
- Repeat this process for each session, and each day you wish to receive credits.

Pest Control Advisor (PCA), Qualified Applicator (QA), Private Applicator (PA)
- Pickup scantron at the start of the day at first session you attend; complete form.
- Sign in and out of each session you attend.
- Pickup verification sheet at conclusion of each session.
- Turn in your scantron at the end of the day at the last session you attend.

Sign in sheets and verification sheets are located at the back of each session room.
AGENDA

• Tim Birmingham, Almond Board of California, moderator
• Jesse Miller, NSF International
• Maria Hoffmann, FDA Center for Food Safety and applied Nutrition
Next Generation Sequencing - The Technology and its Applications - Friend or Foe?

Jesse D. Miller, Ph.D.
Director
Applied Research Center
NSF AuthenTechnologies
Agenda

Next Generation Sequencing

Methods

Applications and Examples
Next Generation Sequencing

Process of extracting genetic material and reading the “code”.
Let's go Back in Time...............1952.
How do we Analyze DNA? Sequencing Background

- RNA sequencing was first to be developed (different methods)

1965: Robert W. Holley sequenced tRNA from *Saccharomyces*

1976: Walter Fiers’ lab first to complete RNA-based genome (MS2 bacteriophage)

Big breakthrough was DiDeoxy Sequencing (Sanger Sequencing)


1977: First to sequence DNA-based genome (PhiX bacteriophage)

Termed the “Chain Termination Method”

Di-Deoxy Nucleotides are labeled with fluorophores. Used to be radiolabeled. Probably mouth pipetted too!

These Di-Deoxy NTPs (ddNTPs) terminate the extension reaction when incorporated via PCR

The end of each fragment has a fluorescent signal

Current method is to run through a capillary gel to size and order

Used to be a polyacrylamide gel

Capture the signal sequence and translate to nucleotide bases
What is Next-Gen Sequencing?

- Term used for sequencing that has a higher throughput than traditional Sanger sequencing
- Now Encompasses many platforms – ThermoFisher, Illumina, Pacific Biosciences, Oxford Nanopore
- **Can be Whole Genome Sequencing, 16S rRNA Metagenomics, Shotgun Metagenomics, Targeted Gene Sequencing, RNA-SEQ**
- 1st Gen – Sanger, ABI (3130xl)
- 2nd Gen – 454, Illumina (Solexa) and ThermoFisher (Massively Parallel Sequencers – Short Read)
- 3rd Gen – Pacific Biosciences, Oxford (Long Read Sequencers)
How is NGS Different than Traditional Sequencing?

- Sequencing done on flowcells/chips now. No 2D gels or capillaries required
- MUCH more data generated (Terabases now, kilobases then)
- Sophisticated BioInformatic programs exist to parse out the data - In some instances, can sequence a sample for around $40
  - MUCH cheaper than historical
- Open source data sharing for massive datasets
- Cloud computing capability

MORE DATA
CHEAPER PER BASE
INTERNET MAKES COMMS AND ANALYSIS EASY
Choose the Right “Fit for Purpose” Tool for the Job
Pulse-Field Gel Electrophoresis (PFGE)

- "Gold Standard" of bacterial DNA fingerprinting
- Restriction enzymes cut bacterial DNA in specific locations
- Multi-directional gel electrophoresis produces unique pattern based on the fragment sizes
- Allows Comparisons between organisms for ID
- Not quantitative
- $100-260
Polymerase Chain Reaction

Semi Quantitative (With a standard curve)

Targets a region of genome for amplification

- Positive reaction = gene is present

Can detect several target genes at once (Multiplex)

Cheap! $5-10/reaction

Several hours to run
Immunological Methods

ELISA
Lateral Flow
Cheap ($5-10)
Fast
Yes/No answers

- ELISA can be quantitative
What is Whole Genome Sequencing?

- Whole Genome Sequencing is the term used for extraction of DNA from an organism and the subsequent mapping of its genome.

- The genetic code (AGCT) is read on an instrument and written into a digital file.

- That digital file can be assembled (like a linear puzzle) to determine the order of the code in the organism.

- Once you have the ordered code, you can analyze the data and make comparisons and data-driven decisions about the organism.

- Not quantitative

- $50/sequence – Up to $500 for assembly/closure (Bacteria)
What is 16S/Shotgun Metagenomics?

- 16S Sequencing on Next-Gen platforms follows a similar workflow, except that it targets a specific gene (16S Ribosomal RNA) used to identify bacteria.
- Metagenomics applies this concept to mixed consortia, resulting in a profile of bacteria abundance (e.g., your microbiome).

1. Sequence DNA
2. Alignment to reference databases. Classifying unknown bacteria into taxonomic groups
3. Visualize in phylogenetic trees, pie charts, or other analyses based on question to be asked

POPULATIONS
NGS Benefits Over Other Methods Moving Forward?

- NGS differentiation (resolution) is unmatched
- With high-throughput efficiencies, NGS is cheaper and faster
- NGS enables much more in-depth data analysis, such as functional genes and heredity
- Cost will continue to decrease
- Database availability and power will continue to increase
- Global adoption and data sharing will increase value
Applications and Examples
Whole Genome Sequencing

What can I use it for?

- Epidemiology
- Resistance
- Strain level ID
- Authenticity

- MUCH deeper look into genome than Pulse Field Gel Electrophoresis or RFLP
- Looking at every base, not just where enzymes cut
Strain Level ID

- Is there value in knowing who your resident strains are?
  - Can you eradicate them more easily?
  - Can you modify processes and cleaning regimens?

- Proactivity?
  - Value in transparency and ownership?
    - Working toward a positive solution

- Third Party sequencing
  - Metadata housed by third party
Speciation of Campylobacter

Commensal bacteria on Chicken and other fowl

Interventions can knock down numbers, but hard to completely eradicate

Three strains under scrutiny

- Jejuni
- Coli
- Lari
Food Pathogen ID – Off the Shelf

Isolate germs from off the shelf meats
Assay for pathogens
Whole Genome Sequence for species

- Common trends?
  - Food type
  - Geography
  - Intervention method
  - Preservation method
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POPULATIONS
Irrigation Water Microbiome

Looking at changes in water microbiome when E. coli present
  - Searching for markers of contamination

Almond Harvest
  - Shaking trees to release fruit
  - Drying for a few days
  - Harvester
  - Hulling
  - Shelling

Source: CA DWR. California Water Plan Update 2013.
Hospital Microbiome

Looking at environment and patient colonization
- Searching for correlations to understand flora

Proactive Treatment?
- Our microbiome protects us – keeps the bad players out
- Antibiotics kill our normal flora
- Probiotic treatment can prevent undesirable bugs from taking hold
- Understanding what is out there allows decision making with more cards in your deck

www.pinterest.com
Epidemiology

- Track and trace your strains
- Look for Single Nucleotide Polymorphisms
  - These changes happen in an organism over time
  - Differentiates one bacteria from another
- GenomeTrakr Application
  - A Database of organisms that can be mined to determine source and traceback
  - Publicly available!
Next Generation Sequencing – Friend!

Sequencing is the Future of Food Safety and Microbial Science

Not Scary! Just a way to get detailed information about the organism you are analyzing

- Can use genomic info to understand
  - Resistances
  - Phylogeny
  - Pathogenesis
  - Epidemiology
- Authenticity
- Make Data Driven Decisions
- Sensitivity – Better Decisions, Faster. Saves lives!
Next Generation Sequencing – The Technology and its Applications – Friend!

Jesse D. Miller, Ph.D.
JDMILLER@NSF.ORG
734.707.5413
Technology in the Food Safety World: Whole Genome Sequencing—friend or foe?

The Almond Conference
Sacramento California
December 5, 2017

Maria Hoffmann, Ph.D.
Genomics Research Microbiologist
Tracking contamination down...and FAST!

Global Point Source

Import Lines

Ecologic Reservoirs

Finished Product

Processing Facility

Farm

SAVES LIVES
Some perspective on the food supply

• Tracking and Tracing of food pathogens
  • Almost 200,000 registered food facilities (2/14)
    • 81,574 Domestic and 115,753 Foreign
  • More than 300 ports of entry
  • More than 130,000 importers and more than 11 million import lines/year
  • In the US there are more than 2 million farms
The Complex Etiology of Foods

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
Gold standard method for pathogen identification

PFGE: banding patterns determine discrimination within serovar.

PulseNet, est. 1996
http://www.cdc.gov/pulsenet/
PFGE v/s WGS

• **WGS is high resolution**
  3-5 million data points are collected for each isolate

• **WGS analyses are statistically robust**
  Unlike PFGE patterns, WGS data can be analyzed in its evolutionary context.
  Accurate and stable genetic changes within pathogen genomes enable us to pin point specific common sources of outbreak strains (farms, processing plants, food types, and geographic regions)
Pedigree vs Phylogeny
DNA based pathogen surveillance not new

- **Flu**: 1990s – flu vaccines predicted from phylogenetic trees
- **HIV**: 1990s – early tracking of HIV transmission using phylogenetics

http://evolution.berkeley.edu/evolibrary/news/081101_hivorigins
CDC investigated a multistate (29 states) outbreak

- 410 confirmed cases between January 1st and July 7th, 2012
- Among the 326 case patient, 55 (17%) had been hospitalized
- Yellowfin tuna was implicated as source of this outbreak
- This product had been imported from an Indian corporation and was used to make spicy tuna sushi for restaurants and grocery stores
- At this time no reference genome was available at NCBI

**Salmonella enterica serovar Bareilly**
PFGE identical in red

NGS distinguishes geographical structure among closely related *Salmonella* Bareilly strains
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**Different PFGE than the outbreak pattern**

**Same PFGE but not part of the MD isolates – in green**

**Outbreak Isolates – in purple**
• Same PFGE cluster together (120 SNPs)
• Outbreak isolates cluster together with 100% bootstrap
• Closest neighbor differ by 20 SNPs
2-part paradigm shift

1. **Whole genome sequencing**
   - High resolution data
   - Harness established field of evolutionary theory for analyses

1. **Open data**
   - Raw genome sequences made available to the public 1-2 days after collection
   - Data made public *before* FDA analyses are performed
Why Develop a WGS Based Network?

• Tracking and Tracing of food pathogens
  • Insufficient resolution of current tools
    - matching clinical to environmental
  • Faster identification of the food involved in the outbreak
  • Limited number of investigators vs. facilities and import lines
• Global travel
• Global food supply
Basic Data Flow for Global WGS Public Access Databases

DATA ACQUISITION
Sequence and upload genomic and geographic data

DATA ASSEMBLY, ANALYSIS, AND STORAGE
International Nucleotide Sequence Database Collaboration (INSDC)
Shared Public Access Databases
- NCBI – National Center for Biotechnology Information
- EMBL – European Molecular Biology Laboratory
- DDBJ – DNA Databank of Japan

PUBLIC HEALTH APPLICATION AND INTERPRETATION OF DATA
- Find clinical links
- Identify clusters
- Conduct traceback
- Develop rapid methods
- Develop culture independent tests
- Develop new analytical software

Other distributed sequencing networks
INSDC
NCBI, EMBL, DDBJ

11/2014
State, Local, Federal, and Foreign Public Health Agencies
Academia/Industry
FDA’s GenomeTrakr

• Distributed network of labs to use whole genome sequencing

• Contributing members:
  • 13 FDA labs
  • 11 PulseNet labs (state public health labs)
  • 5 Dept. of Agriculture labs
  • 7 University labs
  • 1 U.S. hospital lab
  • 2 international labs (Argentina, Mexico)
  • 3 private contracting labs

• Data curation and bioinformatic support/analyses provided by National Center for Biotechnology Information (NCBI) and FDA-CFSAN.
GenomeTrakr verses PulseNet?

food/env -> GT

clinicals -> PN

food/env -> GT

clinicals -> PN

NCBI
Database growth:
Currently: over 150,000 genomes (all contributors)
Publicizing data

**NCBI:**

**Sequences and metadata**
- fastq files in SRA DB, annotated assemblies in GenBank
- metadata in BioSample DB (taxonomy, collected by, country and state, year, isolation source)
- **Private**: city, county, zipcode, firm names, product names, patient data (age, sex, etc)

**Analyses**
- Phylogenetic trees for each pathogen published daily at NCBI: 

**GitHub:**
Pathogen Detection

NCBI Pathogen Detection integrates bacterial pathogen genomic sequences originating in food, environmental sources, and patients. It quickly clusters and identifies related sequences to uncover potential food contamination sources, helping public health scientists investigate foodborne disease outbreaks.

Find isolates now!

Explore the Data

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<tr>
<th>Species</th>
<th>New Isolates</th>
<th>Total Isolates</th>
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<td>Salmonella enterica</td>
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<td>E.coli and Shigella</td>
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<td>Campylobacter jejuni</td>
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<td>12,818</td>
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NOVEMBER 15, 2017

Learn More
- About
- FAQ
- Factsheet
- Antimicrobial Resistance
- Contributors

Data Resources
- Isolates Browser
- Antimicrobial resistance reference gene database
- Isolates with antibiotic resistant phenotypes
- Beta-lactamase resources

New isolate check - *Salmonella*

| # | Organism Group | Strain | Serovar | Isolate | Create D | Location | Isolation f | Host | SNP cluster | Min-same | Min-diff | Sample | Assembly | K-mer group | AST phenotypes | AMR |
|---|----------------|--------|---------|---------|----------|----------|------------|-------|-------------|----------|----------|--------|----------|------------|------------|-------------|----------------|-----|
| 1 | Salmonella enterica | FSIS117 | Saintpaul | PDT000262673 | 2017-11-08 | USA:NJ | Product- Raw- Ground, Communitite or Otherwise Nonintact- Pork | environment: | PDSS000004163 | 2 | 1 | SAMN08000024 | | | | | |
| 2 | Salmonella enterica | OH-17- 19345 | Typhimur | PDT000262757 | 2017-11-09 | USA:OH | Sus soroga domestica | environment: | PDSS00013843 | 9 | 2 | SAMN08007296 | | | | | |
| 3 | Salmonella enterica | OH-17- 18027 | Heidelberg | PDT000262754 | 2017-11-09 | USA:OH | Bos taurus- Pakistan | environment: | PDSS000000264 | 4 | 4 | SAMN08007295 | | | | | |
| 4 | Salmonella enterica | OH-17- 18627 | Dublin | PDT000262762 | 2017-11-09 | USA:OH | Bos taurus- Lung | environment: | PDSS0000002385 | 10 | 9 | SAMN08007291 | | | | | |

SNPs distance to same category

SNPs distance to different category
Look at close matches within SNP cluster
## Pathogen: environmental/food/other sample from Salmonella enterica subsp. enterica serovar Typhimurium

### Identifiers
- **BioSample**: SAMN08007206; SRA: SRS2676514; CFSAN: CFSAN071040

### Organism
- **Salmonella enterica subsp. enterica serovar Typhimurium**
  - Cellular organisms: Enterobacteriaceae; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Salmonella; Salmonella enterica; Salmonella enterica subsp. enterica

### Package
- **Pathogen: environmental/food/other: version 1.0**

### Attributes
- **geographic location**: USA/OH
- **latitude and longitude**: missing
- **strain**: OH-17-19345
- **isolation source**: Sus scrofa domesticus-Feces
- **isolate name alias**: CFSAN071040
- **collection date**: 2017
- **collected by**: Ohio ADDL
- **serovar**: Typhimurium
- **sub species**: enterica
- **PublicAccession**: CFSAN071040
- **ProjectAccession**: PRJNA338674
- **Species**: enterica
- **Genus**: Salmonella
- **attribute_package**: environmental/food/other

### Links
- **BioProject**: PRJNA338674 Salmonella enterica
- Retrieve all samples from this project

### Submission
- **CFSAN**: 2017-11-09
- **Accession**: SAMN08007206 ID: 8007206
- **BioProject**: SRA
### AMR genotype prediction

<table>
<thead>
<tr>
<th>#</th>
<th>SRA Center</th>
<th>Organism Group</th>
<th>Strain</th>
<th>Sex</th>
<th>Source</th>
<th>Create Da</th>
<th>Location</th>
<th>Isolation</th>
<th>Isolation type</th>
<th>Host</th>
<th>SNP cluster</th>
<th>Min-ei</th>
<th>Min-di</th>
<th>BioSample</th>
<th>Assembly</th>
<th>K-mer group</th>
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<th>AMR genotypes</th>
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<tr>
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<td>Salmonella enterica</td>
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<td>aapH(3)-1d</td>
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How do we use the GenomeTrakr information?

- Identify SNP cluster of interest from NCBI
- Download raw data AND run CFSAN SNP pipeline inhouse
What happens with a WGS link between a clinical and environmental sample?

• **Likely result in the following steps:**

  (1) facility/farm inspection and sampling

  (2) Pathogen positive samples are sequenced and submitted to the database

  (3) traceback/trace forward of raw materials and finished product

  (4) WGS is powerful tool that supports investigation
Salmonella 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)
Comparing Traditional and Retrospective Outbreaks in Nut Butters

**Traditional Outbreak Investigations**

- *Salmonella* Typhimurium (Company B, Brand B Peanut butter, 2008/2009): 714 cases, 166 hospitalizations, 9 deaths
- *Salmonella* Bredeney (Company C/Brand C Peanut butter, 2012): 42 cases, 10 hospitalizations, 0 deaths

**Retrospective Outbreak Investigation**

- *Salmonella* Braenderup (Company D/Brand D nut butter, 2014): 6 cases, 1 hospitalization, no deaths
Timeline for Traditional Approach to Foodborne Illness Investigation

- Contaminated food enters commerce
- Identify illnesses and get PFGE pattern from clinical samples
- Identify contaminated food and confirm that product or environmental sample PFGE pattern matches the clinical sample pattern
- Source of contamination identified too late to prevent most illnesses
Timeline for Foodborne Illness Investigation Using Whole Genome Sequencing

Contaminated food enters commerce

Source of contamination identified early through WGS combined database queries

Averted Illnesses

Days

Number of Cases

FDA, CDC, FSIS, and States use WGS in real-time and in parallel on clinical, food, and environmental samples
Immediate benefits of WGS to industry, growers, and distributors

• Earlier intervention means:
  1) Reduced amount of recalled product;
  2) fewer sick patients
  3) less impact overall and minimal damage to brand recognition.
The Fresh-cut Tomato Supply Chain is complex
WGS-based monitoring can pinpoint root causes
Example 1

Field 1

Field 2

Processing facility
Example 2

Field 1

Field 2

Processing facility
Benefits to industry, growers, and distributors (continued)

• Regular testing throughout network:
  1) identifies specific suppliers that are introducing contaminants;
  2) identifies whether contaminant is resident to a facility or transient;
  3) knowledge of where contaminant is coming from allows industry to fix the problem based on scientific evidence.
    • Shift costs to the supplier who has introduced the contaminant.
    • How often is the root cause of the problem left unresolved to occur again at a later date?
One Data Record - Many Possibilities

- SNP
- Markers
- Serotype
- wgMLST
- Virulence

.....AAGCTTGGAGATCTACGTGTACCTAGTCGAAGACTGAGGCTCTA....

- Biofilm persistence
- Resistance (Disinfectant, Heat, Heavy metal...)
- Ecological Fitness
- Adaptation
- Unknown

(Disinfectant, Heat, Heavy metal...)

Adaptation
Improving Food Safety

1. Identify source of foodborne outbreaks more quickly
   ~ WGS provides an integrated food safety surveillance system
   ~ permits international capacity building through integration of foreign food safety entities into the GT network

2. Transparency of open data gives industry full access
   ~ Genome data made public in real-time
   ~ Public software and analysis tools readily available to industry for viewing of results

   ~ WGS compliments rapid testing methods with environmental monitoring for repeat positives and problems w/ resident pathogens.
Genomics of foodborne pathogens for microbial food safety
Marc W Allard, Rebecca Bell, Christina M Ferreira, Narjol Gonzalez-Escalona, Maria Hoffmann, Tim Muruvanda, Andrea Ottesen, Padmini Ramachandran, Elizabeth Reed, Shashi Sharma, Eric Stevens, Ruth Timme, Jie Zheng and Eric W Brown

Whole genome sequencing (WGS) has been broadly used to provide detailed characterization of foodborne pathogens. These genomes for diverse species including Salmonella, Escherichia coli, Listeria, Campylobacter and Vibrio the evolutionary history of these pathogens. Phylogenetics is a powerful tool used for many applications in foodborne outbreak detection and source tracking [1**,2**,3**]. The field started with case studies of past outbreaks demon-
Acknowledgements

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  • Center for Food Safety and Applied Nutrition
  • Center for Veterinary Medicine
  • Office of Regulatory Affairs

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  • Michigan
  • Minnesota
  • New Mexico
  • New York
  • North Carolina
  • Ohio
  • Penn State
  • South Dakota
  • Texas
  • Virginia
  • Washington

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  Eric Brown
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  Maria Sanchez-Leon
Thank you!
Use #AlmondConf to be part of the conversation on Facebook and Twitter.
What’s Next

Tuesday, December 5 at 4:15 p.m.

- State of the Industry – Hall C

Be sure to join us at 5:30 p.m. in Hall A+B for Dedicate Trade Show Time and Opening Reception, sponsored by The Bank of Stockton