WGS Challenges and Opportunities

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Driscoll’s of the Americas
The T cell antigen receptor

Antigen combining site
Carbohydrates
Cytoplasmic tail
Transmembrane region

Vα Vβ
Cα Cβ

Hinge

Resembles an Ig Fab fragment

Domain structure: Ig gene superfamily
Monovalent
No alternative constant regions
Never secreted
Heterodimeric, chains are disulfide-bonded
Very short intracytoplasmic tail
Positively charged amino acids in the TM region
Antigen combining site made of juxtaposed Vα and Vβ regions
30,000 identical specificity TcR per cell
“Everything should be made as simple as possible. But not simpler.”

Albert Einstein
Should Industry use WGS?

- Isolate
  - Internal investigation
    - Cracked floor
  - Database
    - Traffic
    - Penalty
Control measures – cooling and packing

- Protection and handling of primary and secondary packaging
- Hygienic design and cleanliness of cooling tunnels and storage areas
- Movement of people and materials
- Cleanliness of transport equipment
- Facility pest control
Sample “59” was not received.

Numbers correspond to location places indicated by the factory.
Resident or Repeat Transient?

-what is the true ubiquity of sequences?
Direct benefits of WGS / metagenomics for industry

- Characterizing relatedness of strains to assist with investigations
- Understanding factors that contribute to distribution / movement in the environment
- Understanding strain virulence to refine targets of control measures
- Understand mechanisms of entry into plants to design
- Understanding factors that impact persistence
  - Resistance to sanitizing agents
  - Strategies for hygienic design
- Factors that impact survival, biofilm formation
  - Development and validation of control measures
Direct benefits of WGS / metagenomics for industry

• Determine root cause of spoilage
• Improve detection methods and enrichment protocols
• Determine appropriate surrogates for challenge studies
• Further characterize environmental microorganisms to determine most appropriate hygiene indicators
• Assist with detection of food fraud
• Determine health / disease state of plants for agricultural operations
Blueberry mosaic virus
Indirect benefits of WGS / metagenomics for industry

- Characterizing relatedness of strains to assist with source attribution
- Understanding factors that contribute to distribution / movement in the environment
- Understand mechanisms of entry into food plants
- Determine role of microbial populations in nutrition / chronic illness
- Provide inputs for risk assessors to answer specific risk management questions
  - Establishment of FSO, PO, PC
E. coli O157:H7 in lettuce

News about E Coli Lettuce Outbreak 2018
bing.com/news

It may finally be safe to eat romaine lettuce again after a deadly E. coli outbreak sickened people in 32 states

Business Insider 5d
But the CDC said on Wednesday that “the last shipments of romaine lettuce from the Yuma growing region were harv...

CDC comes close to an all-clear on romaine lettuce as E. coli outbreak nears historic l...
The Washington Post 6d

CDC: E. coli outbreak linked to lettuce may be over
News 12 New Jersey 6d

E. coli Outbreak in Canada tied to Yuma Romaine
Food Poison Journal 2d

At A Glance

- Case Count: 172
- States: 32
- Deaths: 1
- Hospitalizations: 75
- Recall: No

People infected with the outbreak strain of E. coli O157:H7, by date of illness onset*
Cyclospora outbreak

592 Cyclospora cases not associated with foreign travel
WGS – Food Safety Management - Considerations

Quality assurance - Standards P- Test

WGS is not just another typing tool - Robust Underpinning Science

Protocol Harmonization Stakeholder Guidelines on Decision-making /Interpretation

Technical competence: authorities, industry, labs, academia

WGS is ONE tool: epidemiology must also be used

Sharing of data: legal, political, psychological constraints

Food safety is global – WGS will be a global tool

Slide: John Donaghy Nestec, SA
Microbiology of the Food Chain — Genomic sequencing of foodborne microorganisms — General requirements and guidance for bacterial genomes

MINIMAL STANDARDS FOR:
Coverage/Data Quality/Chemistry/Alignment Parameters/Data Translation (SNP or Allele Calling)/Clustering Tools/Data Interpretation and Linkage
WGS – It Requires Technical Competencies Beyond Traditional Microbiology

The Whole Genome Sequencing (WGS) Process

WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

Molecular Microbiology

1. DNA Library Preparation
   - Scientists take bacterial DNA and suspend it on a plate with enzymes that break them up into the DNA fragments.
2. DNA Shearing
   - DNAs cut into short fragments of known length, either by using enzymes like “molecular scissors” or mechanical disruption.
3. DNA Library Preparation
   - Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a “DNA library.”
4. DNA Library Sequencing

Bio-Informatics

The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.

‘Wet’ Processing WGS

‘Dry’ Processing WGS

Slide: John Donaghy Nestec, SA
Activity
This activity has two main aims:

• To investigate how NGS applications / research of food and food associated environmental microbial community can contribute to the improvement of risk assessment and risk management options;
• To provide guidance to industry describing values and current limitations on generation of sequence information, bioinformatics analyses and biological interpretation of data employed in NGS analysis.

With the support of ILSI Japan, ILSI North America and ILSI Southeast Asia Region, this activity is tackled at a global scale.
Application of WGS

WGS is infallible (and we won’t need epi anymore).

WGS match between a food and clinical isolate doesn’t mean food caused illness. Epi and traceback critical

What industry heard a few years ago

Current CDC, FDA, FSIS
Challenges for Industry (& others) for Public WGS Databases

- Data Sharing & Implications (Legal/Regulatory)
- Meta Data Required (Confidentiality)
- Data Accessibility (Security)
- Legacy data & Technical Interpretations
Hurdles and challenges

• GenomeTrakr: Access to metadata
• Availability / capacity of sequencing equipment / bioinformatics expertise
• Training and awareness
• Cost vs benefit of technology
• Culture-independent testing?
Opportunities for collaboration

• Specific, targeted studies to answer persistent questions
• Sharing data from facility assessments in a timely manner
• Ecological studies to provide context to sequence data
• Provide awareness, training, support to smaller industry players.