



International Standards Development for Use of Whole Genome Sequencing in Food Microbiology

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FDA-CFSAN
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Impact of NGS on food microbiology

WGS Use Case	Regulatory	Research	Industry
Illness Cluster Detection	X		
Product traceback	X		
Define food adulterant	X		
Source attribution	X	X	
Environmental assessment	X		X
Replace legacy workflows	X		X
Infer origin and evolution of pathogenicity		X	
Determine basis for microbial growth and survival		X	X
Characterize process microbes		X	X
Discover new enzymes, mechanisms, pathways		X	X

Impetus for Global Standardization

- Use of data in regulatory decision-making
- Highly complex and varied workflows
- Reliance on shared datasets
- Lack of consensus on best practices

An internationally-recognized standard would enhance

- Bilateral system recognition
- Basis for 3rd party system audits
- transparency and reproducibility of findings
- Adoption of WGS based analyses and decision-making by public and private sector

Roadblocks to Global Standardization

- Lack of consensus
- Variety of applications (use cases) requiring diversity of approaches
- Complex and proprietary workflows
- Continuing workflow development and improvement

Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) workgroup

Gargas et al., 2012

Table 1 Selected workgroup recommendations for establishing NGS test systems for clinical use

Requirements for test establishment	Objective	NGS-specific recommendations ^a
Validation	Document reliability of the platform, test, and informatics pipeline before testing of patient specimens	<ul style="list-style-type: none"> Platform validation: establish that the system provides reliable sequence analysis across the genomic regions targeted by the test. Test validation: establish that the system correctly identifies disease-associated (and other) variants in targeted regions of the genome (Supplementary Guidelines, section 4). Informatics pipeline validation: establish that the algorithm(s) reliably analyze platform data to produce an accurate sequence. Establish and validate alternate methods (for example, Sanger sequencing) to derive high-quality sequence data for problematic genomic regions.
Quality control	Document reliability of the sequence analysis during patient testing	<ul style="list-style-type: none"> Utilize a combination of QC materials, both intrinsic and/or spiked in, that mimic genomic complexity and the types of mutations the test is designed to detect. During patient testing, quality metrics (for example, quality scores, depth and uniformity of coverage, mapping quality, GC bias and transition/transversion ratio) should be assessed and compared to those established during validation. Clinically actionable findings should be confirmed by independent analysis using an alternate method.
Proficiency testing	The independent assessment of test performance	<ul style="list-style-type: none"> PT challenges should target the analysis of both disease-associated and naturally occurring sequence variations across the genomic regions targeted by the test to measure the reliability of sequence analysis. Electronic sequence files may permit a comparison of alignment and variant calling methods across laboratories but will require additional consideration of platform differences. PT programs should consider the different genomic regions targeted by each recipient laboratory's assays to properly compare inter-laboratory performance.
Reference materials	The use of materials for quality management of the analytical phase of testing	<ul style="list-style-type: none"> RMs with both naturally occurring and disease-associated sequence variations are needed for test validation, QC procedures and the independent assessment of test performance. Synthetic DNA and electronic reference data files may serve as RMs for rare or challenging sequence variations. Efforts should be undertaken to establish a suitable NGS RM and the sequence of the RM should be refined as the technology changes. Such a RM should be annotated to indicate regions of high and low sequence reliability.

^aSee **Supplementary Guidelines** for complete recommendations. RM, reference material.

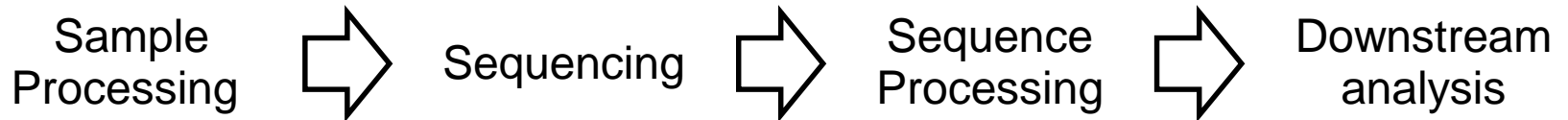
Standards Development for Microbiology

USA	Interagency Collaboration on Genomics and Food Safety(Gen-FS)	Analytical methods, proficiency, training
	NIST	reference materials, analytical methods
	FDA	reference database (ARGOS), NGS process validation
Canada	IRIDA (Integrated Rapid Infectious Disease Analysis)	Metadata (ontology)
Australia	Public Health Laboratory Network (PHLN) expert advisory group	data generation (sequencing), data storage (including backups), data analysis (bioinformatics) and data sharing (or distribution).

Standards development for Microbiology

European Union	COMPARE consortium	Sampling and data collection, sample processing and sequencing, analytical workflows, risk communication tools, evaluating cost effectiveness
	European CDC	Sampling, analytical methods, interagency collaboration
International	Global Coalition for Regulatory Science Research	Analytical methods
	Open Biomedical Ontologies Foundry	Metadata (Controlled vocabularies)
	Global Microbial Identifier (GMI)	Analytical methods, proficiency, metadata
	International Nucleotide Sequence Database Collaboration (INSDC)	Publically accessible database

Scope

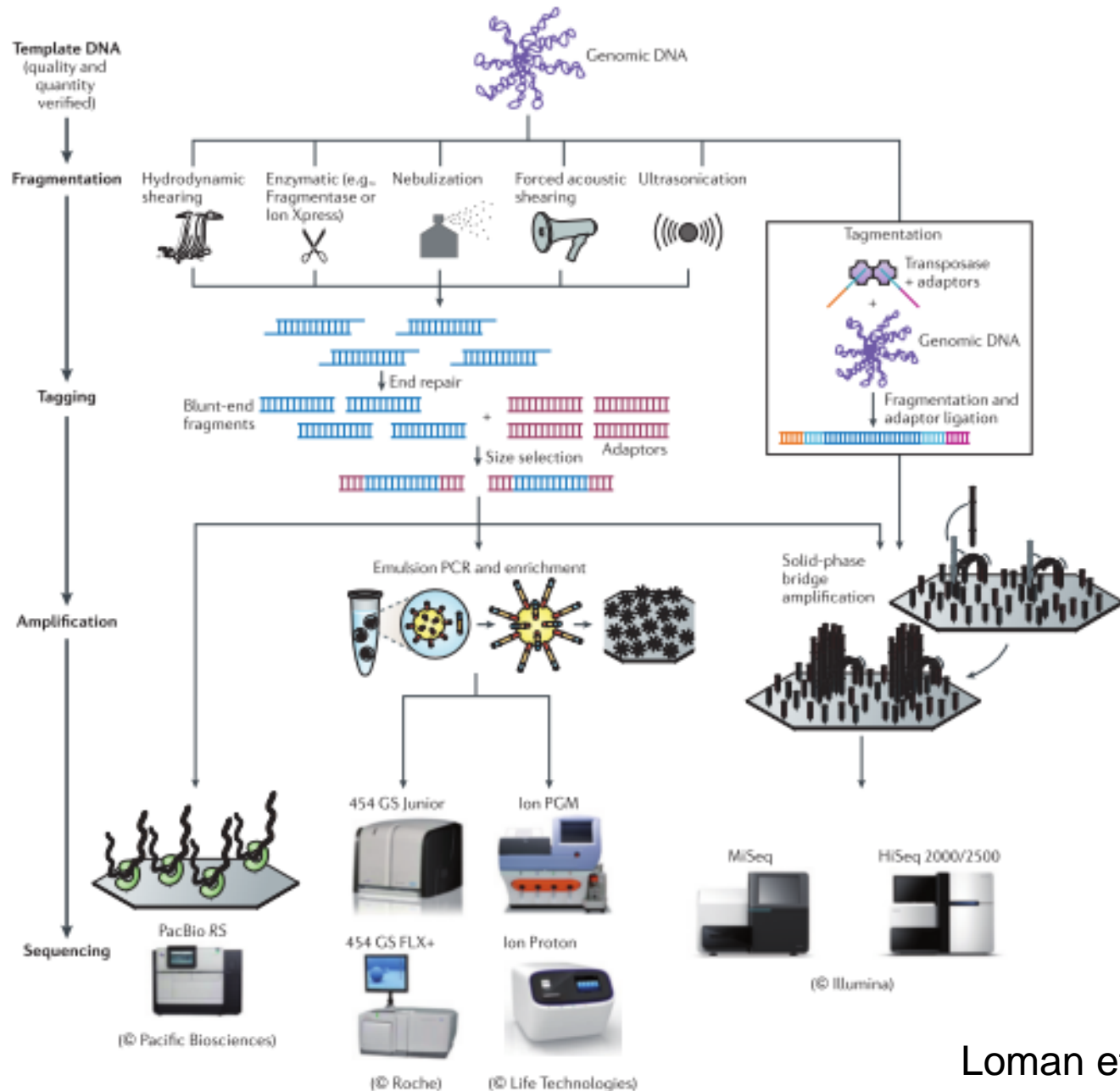


- Culture
- DNA extraction
- Fragmentation
- Tagging
- Amplification
- Enrichment

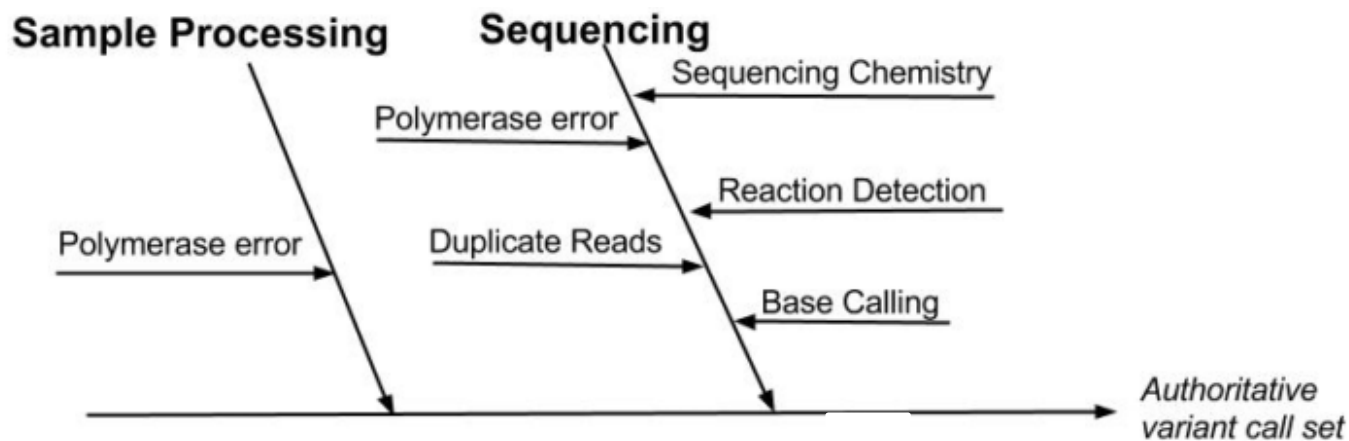
- Data grooming
- Quality control
- Assembly
- Variant Detection and filtering

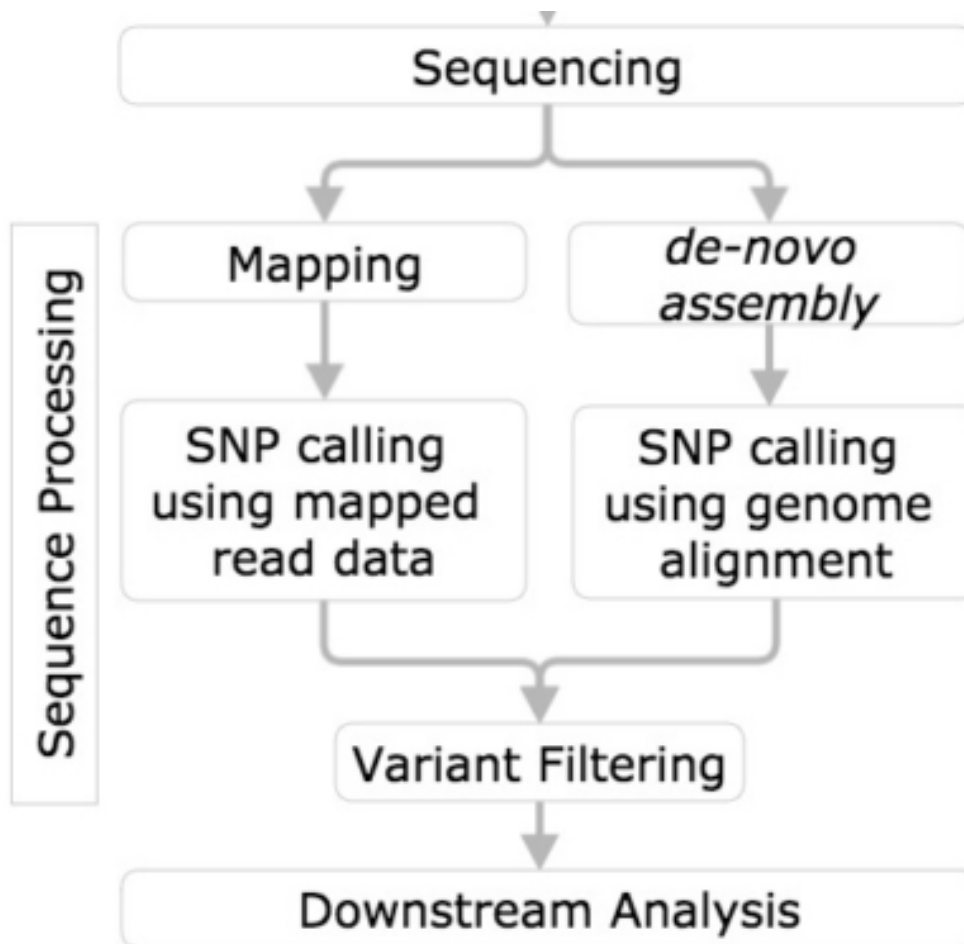
- Share sequence and metadata
- Gene/Allele finding
- Phylogenetic clustering

Platform-specific

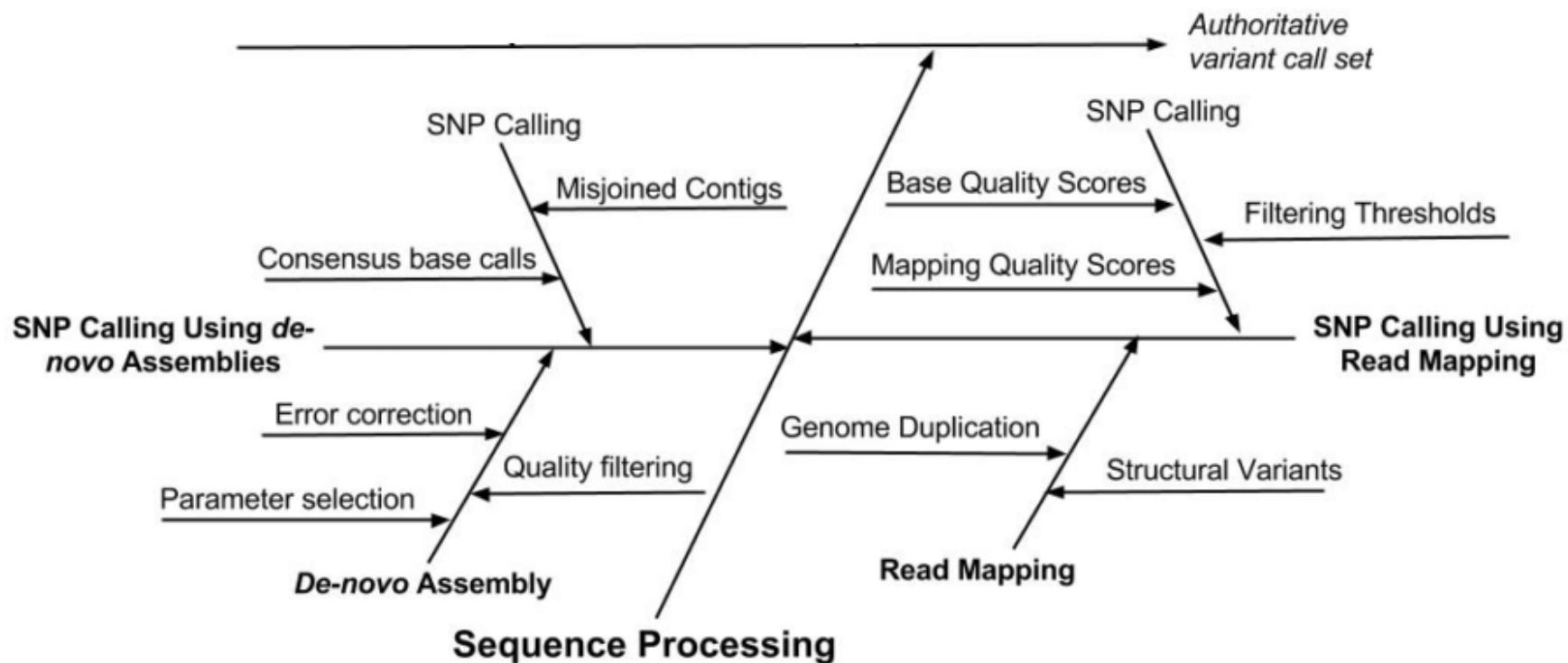


Error during sample processing and sequencing





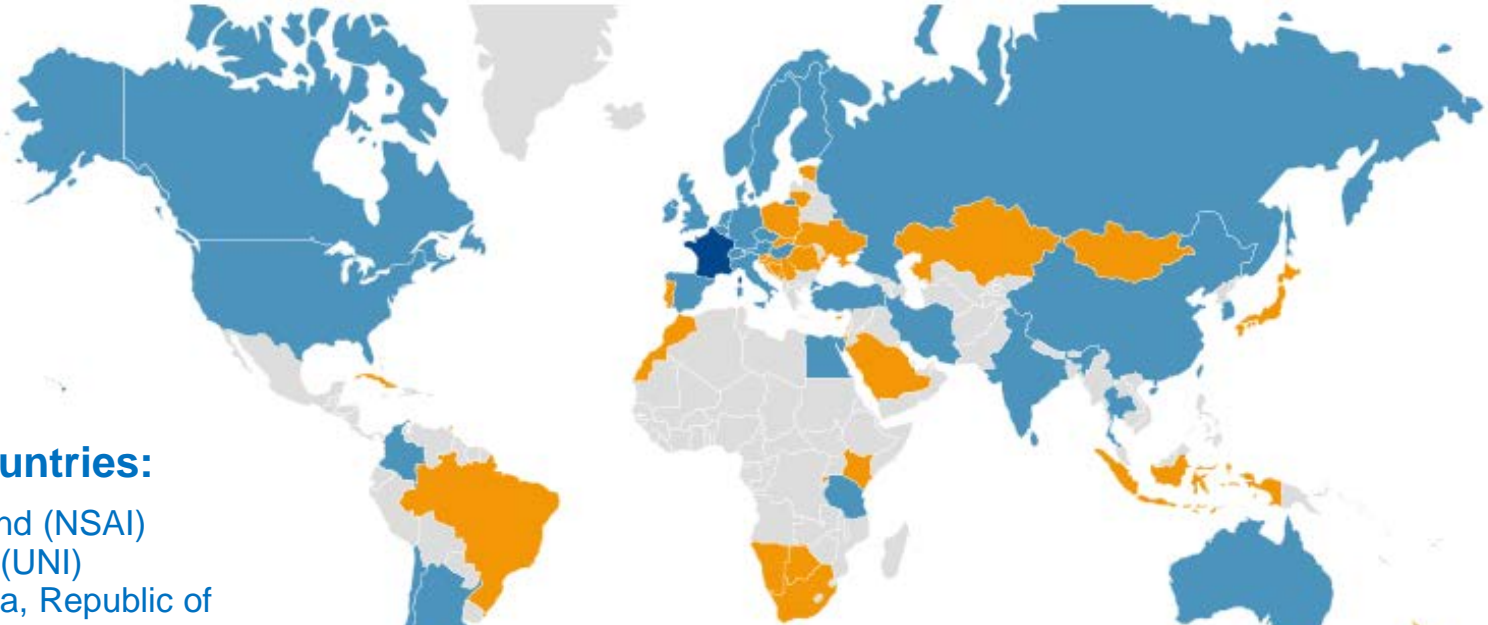
Error during sequence processing



Key principles in ISO standard development



1. ISO standards respond to a **market need**
2. ISO standards are based on **global expert opinion** (*also globally relevant*)
3. ISO standards are developed through a **multi-stakeholder process**
4. ISO standards are based on a **consensus**



Secretariat
France (AFNOR)

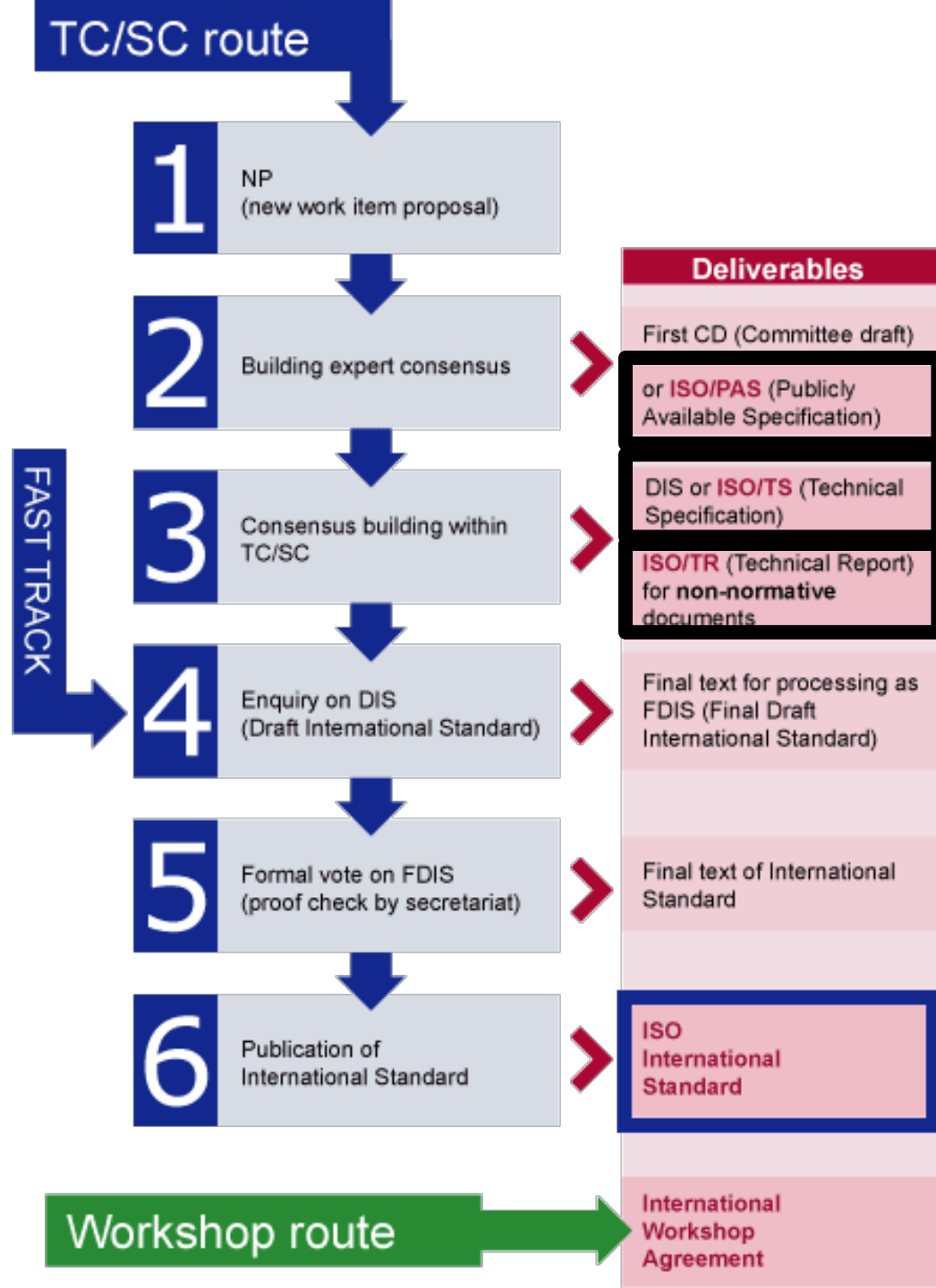
33 Participating countries:

Argentina (IRAM)	Ireland (NSAI)
Australia (SA)	Italy (UNI)
Austria (ASI)	Korea, Republic of (KATS)
Belgium (NBN)	Luxembourg (ILNAS)
Canada (SCC)	Netherlands (NEN)
Chile (INN)	Norway (SN)
China (SAC)	Russian Federation (ANSI)
Colombia (ICONTEC)	Spain (AENOR)
Czech Republic (UNMZ)	Sri Lanka (SLSI)
Denmark (DS)	Sweden (SIS)
Egypt (EOS)	Switzerland (SNV)
Finland (SFS)	Tanzania, United Republic of (TBS)
France (AFNOR)	
Germany (DIN)	
Hungary (MSZT)	
India (BIS)	

30 Observing countries:

Bosnia and Herzegovina (BAS)	Morocco (IMANOR)	Hong Kong (ITCHKSAR)
Botswana (BOBS)	Namibia (NSI)	(Correspondent member)
Brazil (ABNT)	New Zealand (SNZ)	Montenegro (ISME)
Croatia (HZN)	Poland (PKN)	(Correspondent member)
Cuba (NC)	Portugal (IPQ)	
Cyprus (CYS)	Romania (ASRO)	
Estonia (EVS)	Rwanda (RBS)	
Indonesia (BSN)	Saudi Arabia (SASO)	
Israel (SII)	Serbia (ISS)	
Japan (JISC)	Slovakia (SUTN)	
Kazakhstan (KAZMEMST)	Slovenia (SIST)	
Kenya (KEBS)	South Africa (SABS)	
Lithuania (LST)	Trinidad and Tobago (TTBS)	
Mongolia (MASM)	Ukraine (DTR)	

ISO process and deliverables





ISO WG development and activity

Apr 2014	N 861 (CEN)	US Proposal
2014	N1671	<u>Recommendation 370</u> : Standardization work to be launched under ISO SC 9 in collaboration with ISO TC 212 (Clinical Lab Testing and In Vitro Diagnostic Test Systems)
Aug 2014	N1676	<u>Resolution 637</u> (passed Oct 2014): <ol style="list-style-type: none">1. Need for standardization2. Create WG, participating countries, convener3. Mandate WG to discuss topics for standard development and make recommendations to SC 9 Plenary4. For liaison with ISO TC 212
July 2015	N 1779	Call for experts in particular from Asia and also representing food manufacturers and technology developers

WG 25 expert committee membership (Nov 2015)

- 35 members
- Countries represented: Australia, Belgium, Canada, Finland, France, Germany, Ireland, Korea, Netherlands, Switzerland, USA...*no developing (low income) countries*
- Sectors represented: government (18), academic/research institute (8), industry/commerce (7)
- Liaisons
 - to TC 276 WG 3 (Biotechnology)
 - from TC 212 and TC 276 WG 3 (Clinical Lab Testing and In Vitro Diagnostic Test Systems)

New experts are welcome to join WG 25
Contact national standardization body

Vision: To provide a framework for processing NGS data to address global problems in food microbiology worldwide.

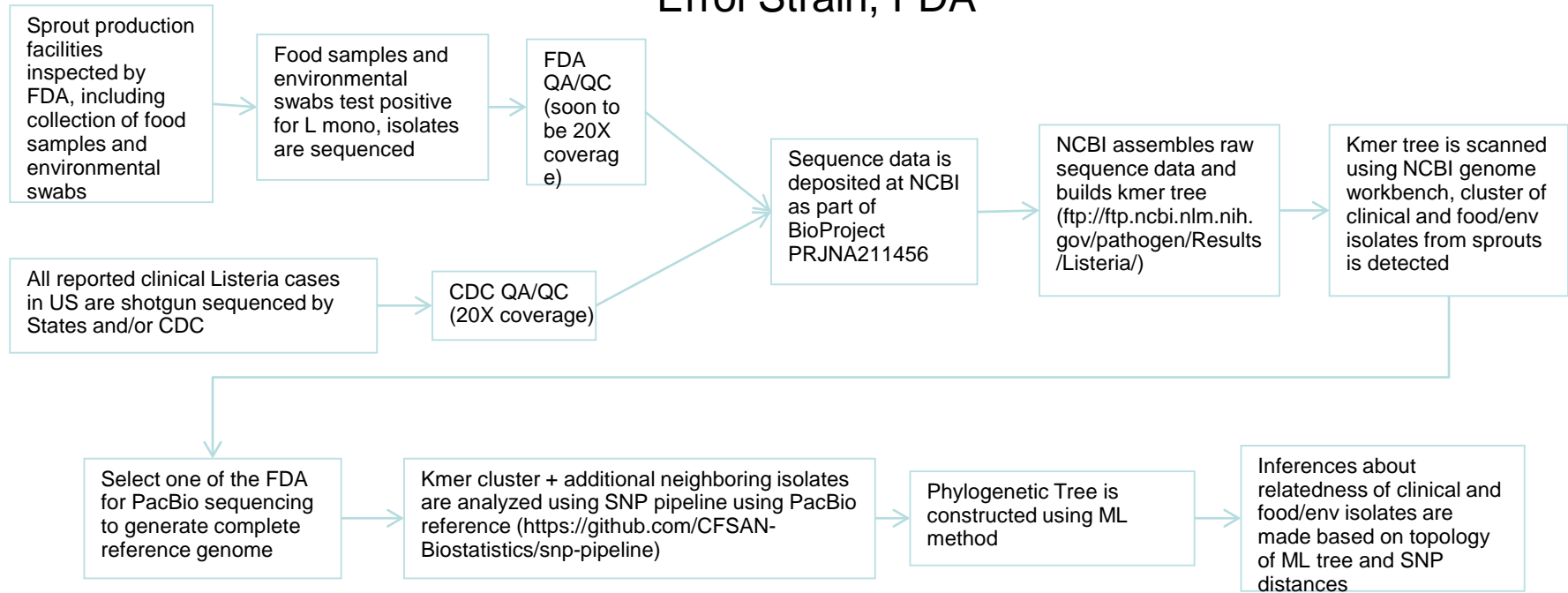
Mission: To develop a consensus among global experts to build and use data models that can accommodate changes in NGS, microbiology, and food processing technology

Other objectives

- Adapt to new platforms and methods
- Readily adaptable to 3rd party audit
- Encourage use of
 - common, non-proprietary formats
 - minimal metadata standards (including controlled vocabularies)
 - publically accessible data

Use Case Scenario: Detection of Product (Sprouts) Contaminated w/ Listeria Causing Illness

Errol Strain, FDA



Desired Outcomes

1. Regulatory Action— Recall, suspension, seizure...
2. Root cause analysis
3. Early detection of outbreak

Liabilities

1. Sample handing (i.e. label mix-ups)
2. Contamination (i.e. false positive in food/env)
3. Restricted access to clinical metadata

Overview of standard outline v1.4 (N16)

<p>Wet lab steps</p>	<p>quality management system, sample tracking, culture receipt and storage, metadata intake and storage, preparation of cultures for sequencing, DNA extraction, library prep, sequencer run statistics, repetition of experiments</p>
<p>Sequence data analysis steps</p>	<p>data grooming procedures, quality metrics and standards, local data storage, demonstration of authenticity, reporting</p>
<p>Validation of data and analytical methods</p>	<p>Analysis of raw sequence, assemblies and phylogenies</p>
<p>Metadata</p>	<p>Public and private metadata, minimal metadata set, validated data entry, controlled vocabularies</p>
<p>Sequence Repository</p>	<p>Availability of transformed data, ability to make corrections, movement of data in and out of repository, data retention, backups and storage</p>



SCOPE

		Yes	No
Target organisms	All foodborne bacteria?		
	Foodborne pathogens?		
	Other organisms (viruses, parasites, fungi)?		
Input material	DNA from culturally isolated organisms (strains)?		
	DNA from non-cultured samples (metagenomics)?		
	RNA from cultured or non-cultured samples?		
NGS platform/technology	Short read?		
	Long read?		
Sequence repository	Private?		
	Public?		

Summary

- ISO Expert WG has been created to consider International Standard in the area of food microbiology
- WG is considering key questions related to scope and developing draft language
- New work item proposal (NP) under development
- Expert WG accepting additional members, especially:
 - Technology developers (laboratory operations and bioinformatic analysis)
 - Food manufacturers interested in using WGS
 - Experts from under-represented areas (Central and South America, Asia, Africa)