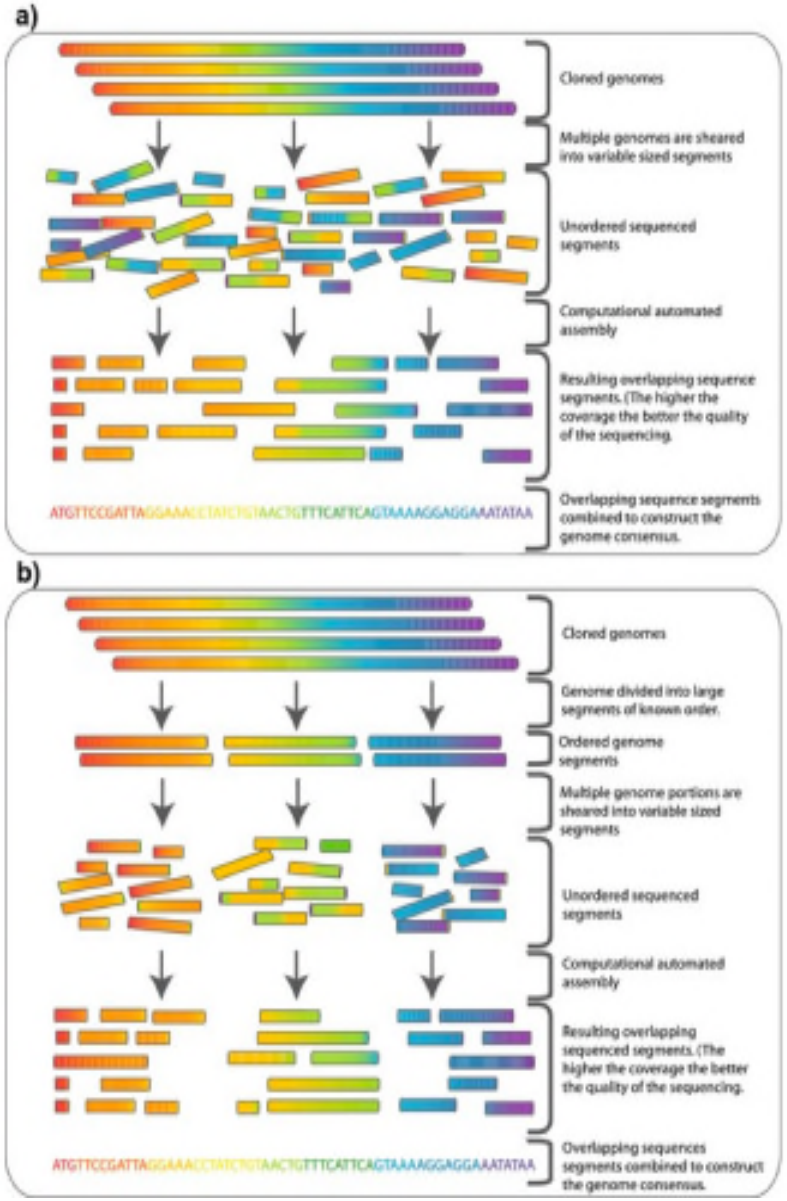

Netherlands Food and Consumer Product Safety Authority
Ministry of Economic Affairs

NL progress on and EFSA's role in Whole Genome Sequencing (WGS)

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Office for Risk Assessment and Research

Investigation of foodborne pathogens

1. Identification of a risk by characterisation of the pathogen

2. Detection of foodborne outbreaks by linking patient isolates with pathogens isolated from food

3. Risk assessment through identification of virulence factors and antimicrobial resistance genes

Typing of microorganisms

- Phage-typing
- Serotyping
- Pulse-field gel electrophoresis (PFGE)
- Multi-Locus VNTR Analysis (MLVA; VNTR = Variable-Number Tandem Repeat)
- Matrix-assisted Laser Desorption/Ionization (MALDI)

- **Whole Genome Sequencing (WGS)**

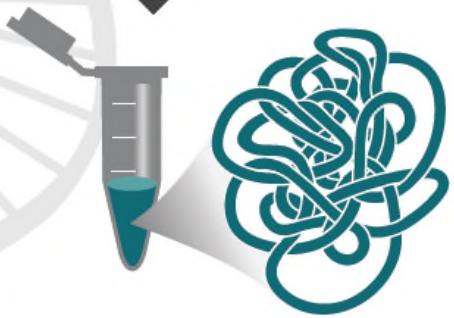
The Whole Genome Sequencing (WGS) Process

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.

Bacterial Culture

1. DNA Extraction

1 Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.



2. DNA Shearing

2 DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.

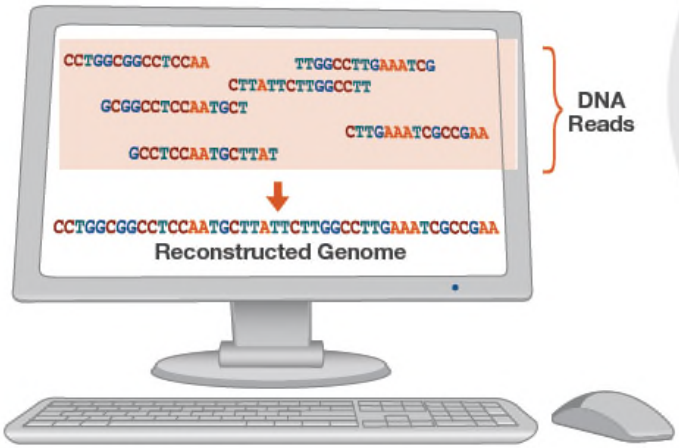
3. DNA Library Preparation

3 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

4 The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."

5. DNA Sequence Analysis



5 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.

WGS

Advantages

- Comprehensiveness
- Costs are still going down
- Older data are not obsolete

Can be used for

- Typing
- Outbreak identification
- Risk assessment

Databases and exchange

- Analysis of WGS data requires comparison of sequences
- Many databases exist with WGS data of pathogens and other microbes
- Exchange of information is not very well organised or regulated
- Non-technical issues such as property issues, jurisdiction, etc., are a major bottleneck for the application of WGS

EFSA's efforts

- In 2012 EFSA concluded a database devoted to WGS is necessary
- The Advisory Forum concluded in 2012 that EFSA should take an active leadership role on WGS
- In 2014 EFSA held a scientific colloquium on WGS food-borne pathogens for public health
- In 2014 the Biohazard opinion recommends establishment of an EFSA-ECDC-EU-RLs platform for utilizing WGS data for public health

Utilization of WGS in The Netherlands

- The National Institute of Public Health and the Environment (RIVM) has decided to apply WGS as the primary tool for typing microbes and has purchased state-of-the-art machines for that purpose

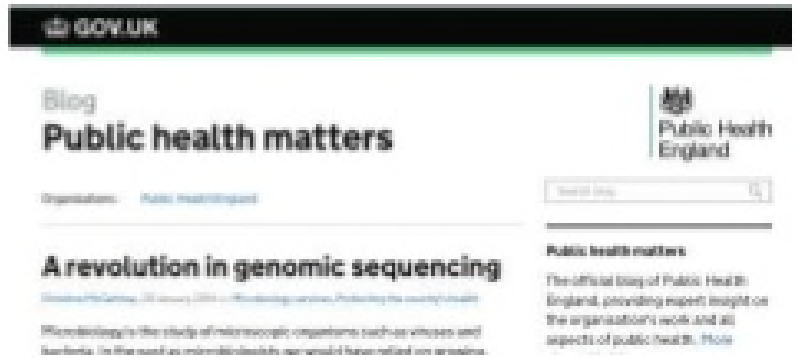
- The Netherlands Food Safety Authority (NVWA) already uses WGS as main typing method for some and soon for all microbes. At the moment samples are sequenced commercially, but a sequencer has been ordered as well for own use

Follow-up EFSA's 2014 recommendations

- EFSA does not use WGS as general typing method
- Developments elsewhere progress rapidly, in particular in the USA & Canada
- National authorities already use WGS and do not want to report PFGE or other type of data
- EFSA has not fully taken up the leadership role

Whole Genome Sequencing of Foodborne Pathogens

- UK Public Health England committed to sequence all the Salmonella isolates submitted to PH Lab
- US FDA and CDC (supported by National Center for Biotechnology Information) created a distributed network of labs to utilize WGS for pathogen identification



https://publichealthmatters.blog.gov.uk/2014/01/20/innovations-in-genomic-sequencing/
http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ucm363134.htm

IRIDA

IMPLEMENTATION OF WGS POLICY: → Foodborne Pathogens

- **Canada is in the process of incorporating information from WGS into Health Risk Assessments and Epidemiological Surveillance**
  - WGS is being performed on, Bacteria, Viruses, and Parasites from Clinical, Environmental, and Food Sources
  - WGS data: currently used to monitor trends in emerging pathogens, AMR, to identify novel virulence factors, and as an parallel/alternative to traditional analyses like MLST and serotyping
  - HC is also working on Quality Assurance and Best Practices Standards Guidelines for sequence analysis (Pightling et al., 2015; 2014).
  - Working toward using WGS to support the development of policy/ standards/compliance.

Recommendations to EFSA

- Allow WGS data for microbiological typing
- Set up WGS databases for foodborne pathogens
- Cooperate with MS authorities on WGS
- Expand and accelerate the cooperation between EFSA and ECDC on exchange of WGS data and establishing databases for WG sequences
- Select WGS as main tool for outbreak investigations of microbial foodborne disease
- Ask the Biohazard panel to perform a self-tasking mandate on WGS