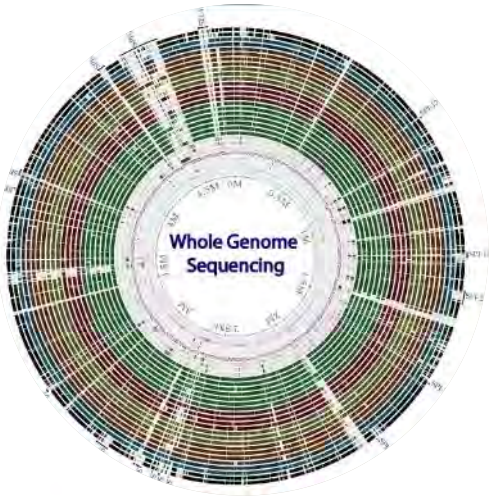
De potentie van Whole Genome Sequencing

Menno van der Voort – Wageningen Food Safety Research



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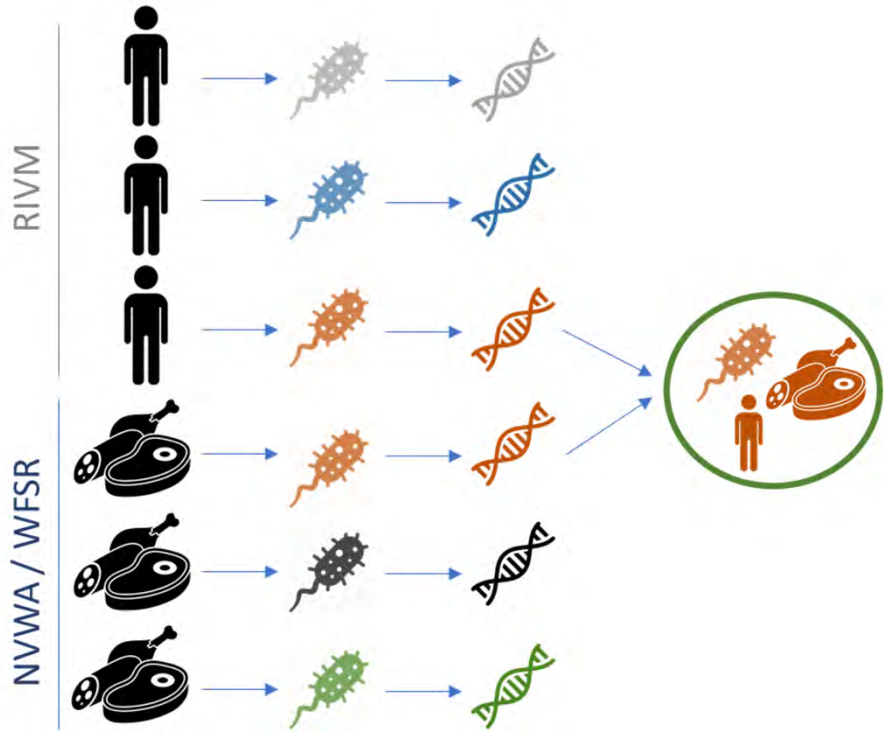
Wat willen we weten?

- Identificatie van bacteriën:
    - Welke bacterie is het?
        - Genus, soort, sub-species, serogroep, sequence type, ...
    - Wat kan deze bacterie?
        - Resistentie, virulentie, enzym productie, antibiotica productie

Surveillance - bronopsporing



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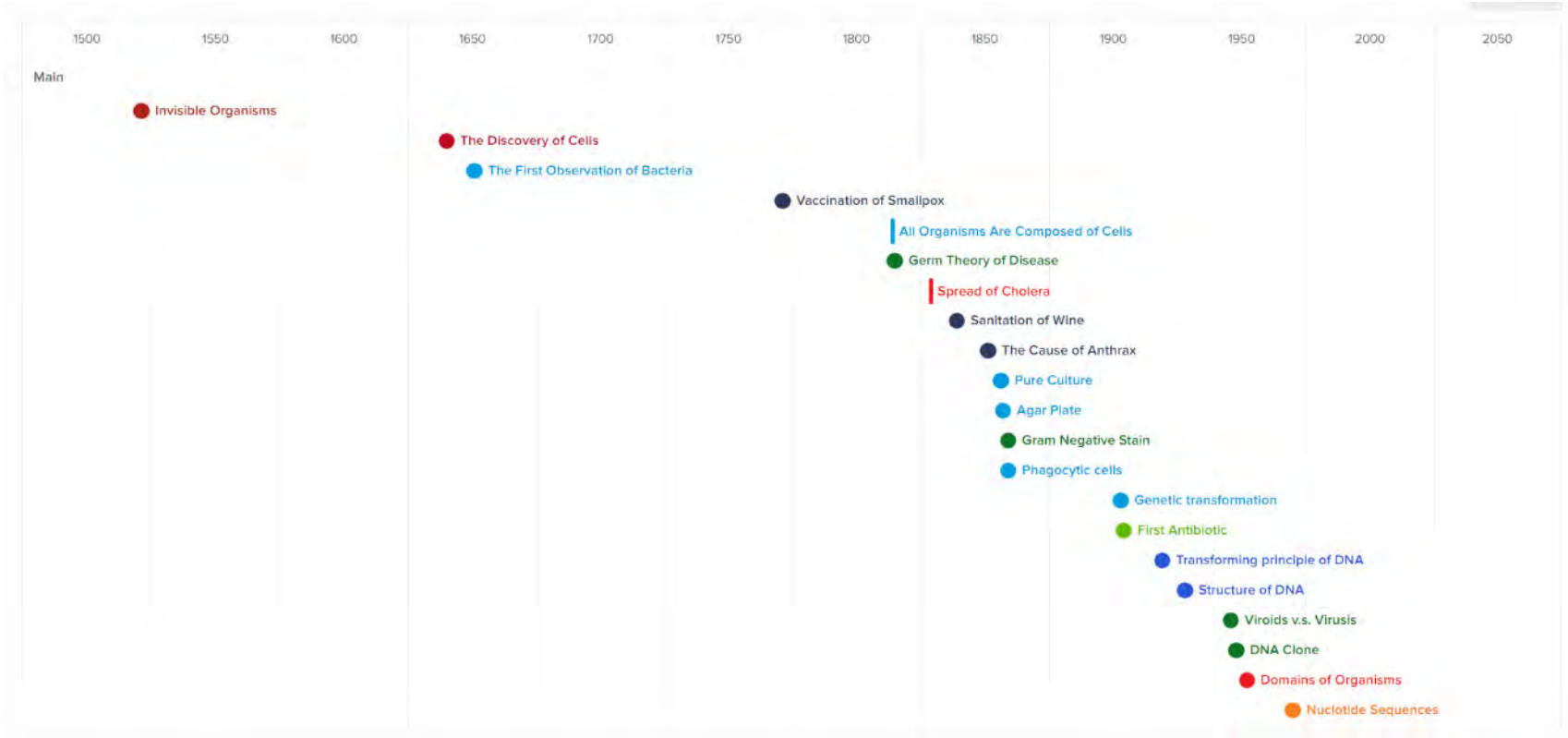
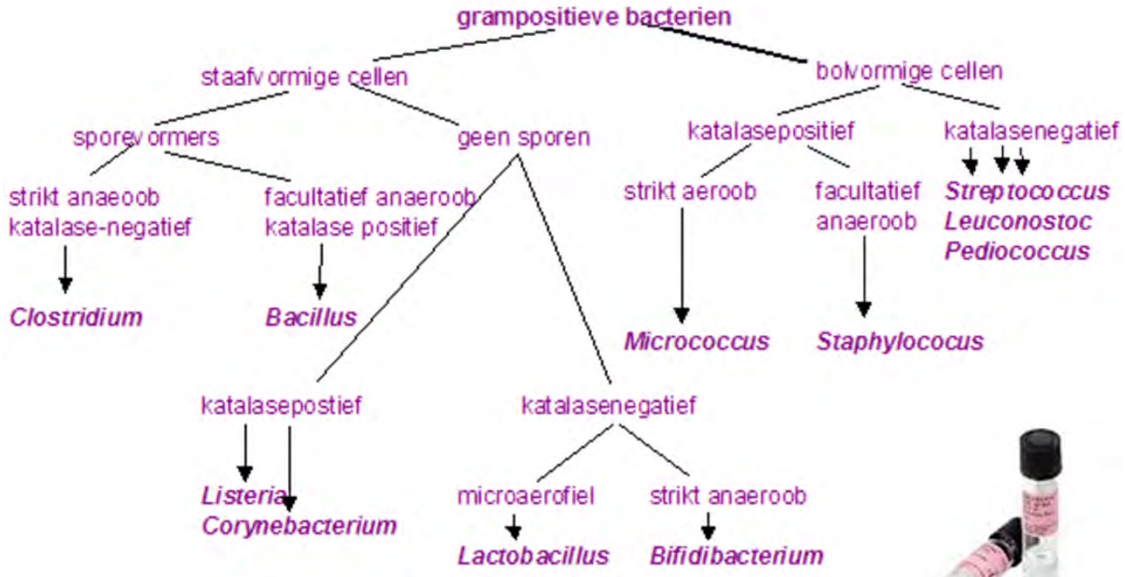
Microbiologie tijdslijn



https://www.preceden.com/timelines/66880-history-of-microbiology

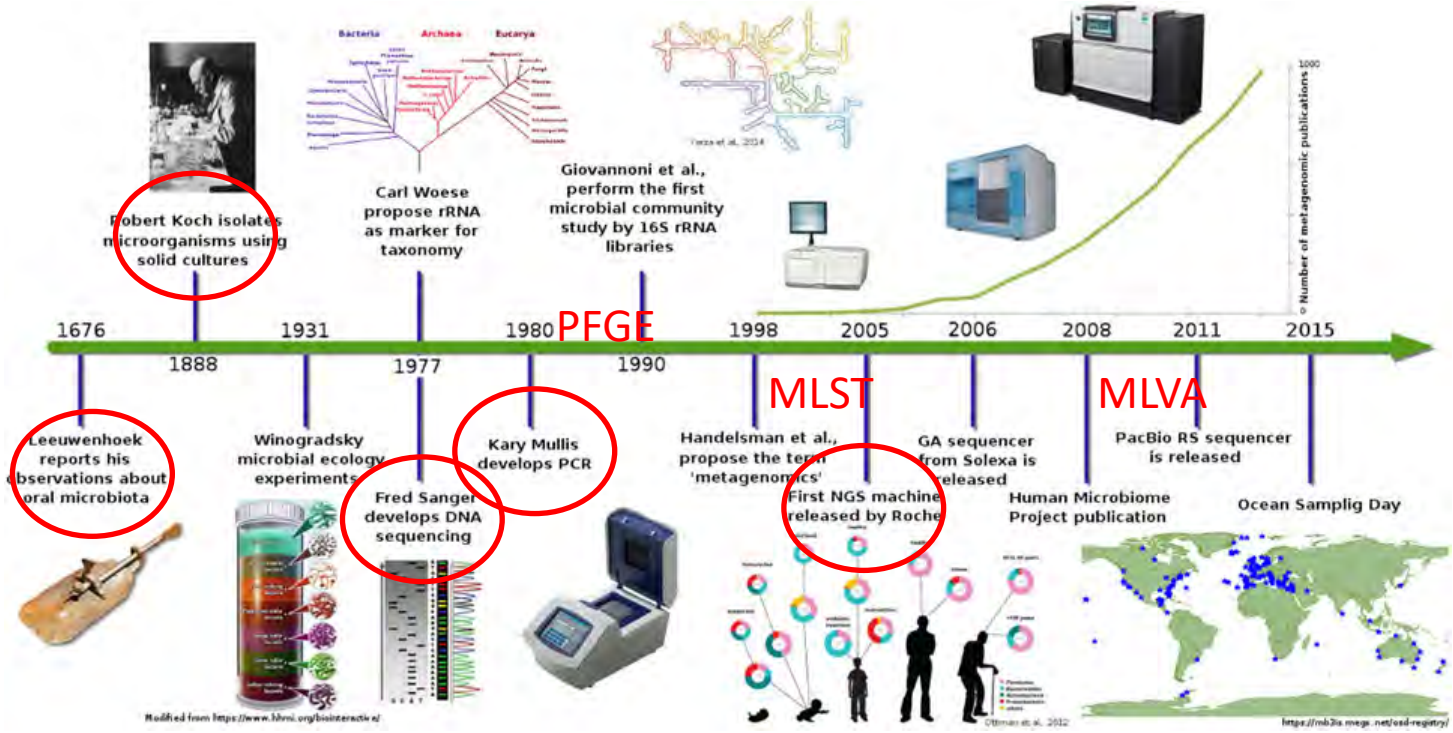
Biochemische typering



MALDI-TOF

https://www.microbiologie.info/

Geschiedenis microbiologie



Metagenomics timeline and milestones. Timeline showing advances in microbial communities studies from Leeuwenhoek to NGS (Ottman et al., 2012; Yarza et al., 2014).

Figuur met dank aan Eelco Franz

Overview typingerings methodes

- Faag-typering → moeilijk te standaardiseren
- Sero-typering → weinig resolutie
- PCR → beperkte informatie

Applicatie → Methode ↓	Surveillance	Evolutie studies	Bron-opsporing	Bron-attributie
PFGE	-	-	+	-
MLST	+/-	+	-	+
MLVA	+	-	+/-	+

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“klassieke” typerings methoden

- *Listeria* - PFGE, serotypering (agglutinatie, PCR), MLST
- *Salmonella* - serotypering, MLVA (Enteritidis, Typhimurium), PFGE
- *Campylobacter* - PFGE, MLST
- STEC - serotypering, MLST, faag-typering, PFGE
- *Staph. aureus* - PFGE, faag-typering

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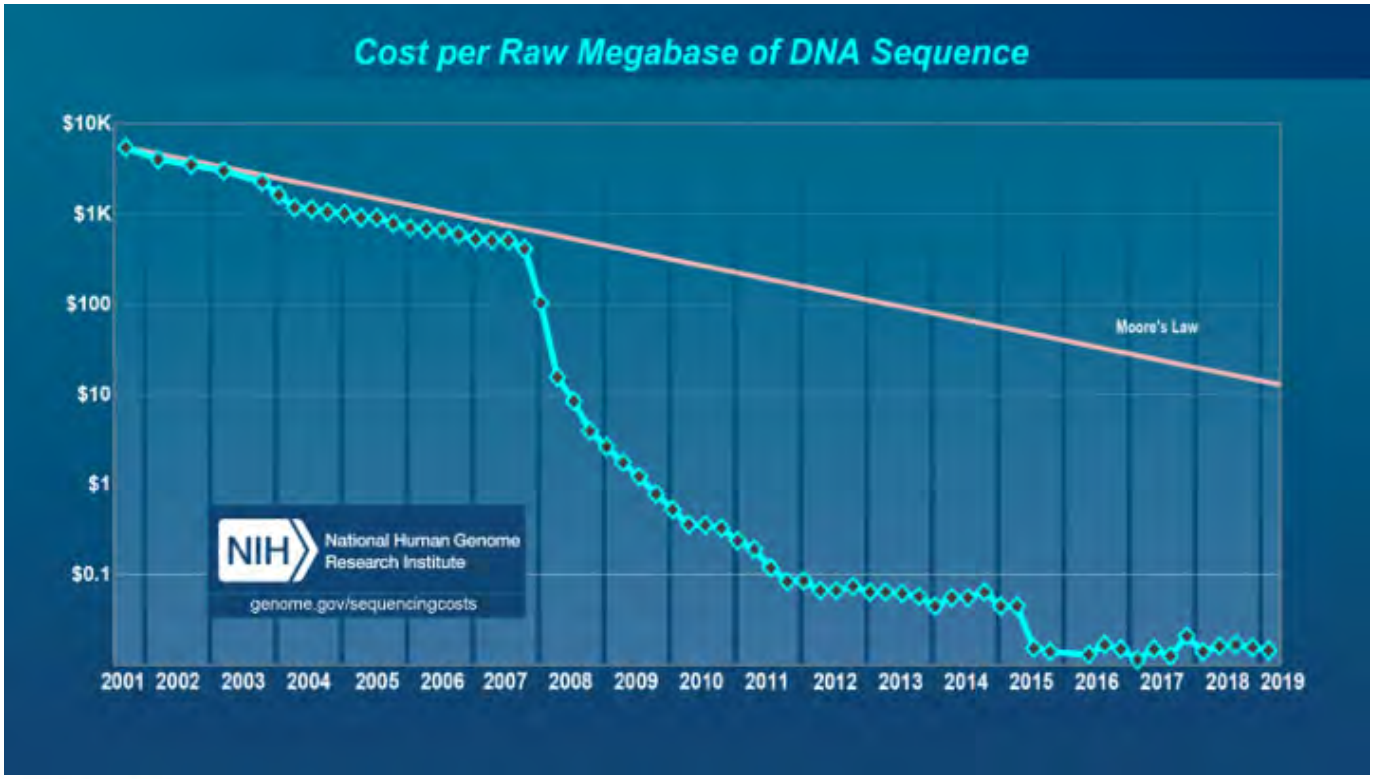
Next Generation Sequencing



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Method	Single-molecule real time sequencing	Ion semiconductor	Pyrosequencing (454)	Se		
Read length	2900 bp average	200 bp	700 bp	50 t		
Accuracy	87% (read length mode), 99% (accuracy mode)	98%	99.9%	98%		
Reads per run	35–75 thousand	up to 5 million	1 million	up t		
Time per run	30 minutes to 2 hours	2 hours	24 hours	1 to dep seq spe leng		
		$1	$10	$0.0		
		Less expensive equipment. Fast.	Long read size. Fast.	Potential for high sequence yield, depending upon sequencer model	Low cost per base.	Long individual reads. Useful for many applications.
Disadvantages	Low yield at high accuracy. Equipment can be very expensive.	Homopolymer errors.	Runs are expensive. Homopolymer errors.	Equipment can be very expensive.	Slower than other methods.	More expensive and impractical for larger sequencing projects.

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Vervanging typingerings methodes door WGS

- Faag-typering → moeilijk te standaardiseren
- Sero-typering → weinig resolutie
- PCR → beperkte informatie

Applicatie → Methode ↓	Surveillance	Evolutie studies	Bron-opsporing	Bron-attributie
PFGE	-	-	+	-
MLST	+/-	+	-	+
MLVA	+	-	+/-	+

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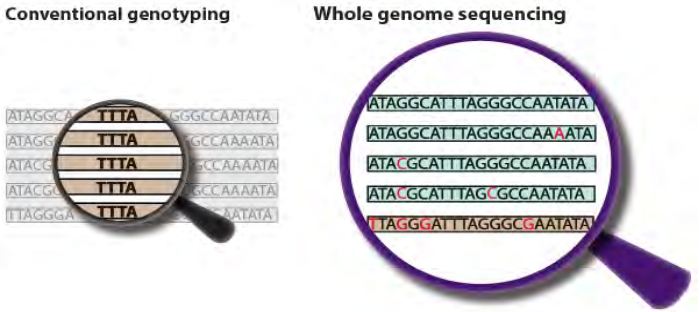
Whole-Genome-Sequencing (WGS)

- Bevat *alle* genetische informatie
  - Serotype
  - Moleculaire typering
  - Virulentie, resistentie profiel

  - Hoogste resolutie voor bronopsporing
  - Toepasbaar op alle bacteriën



Applicatie → Methode ↓	Surveillance	Evolutie studies	Bron-opsporing	Bron-attributie
WGS	+	+ +	+ +	+ +

- Kosten efficiënt??

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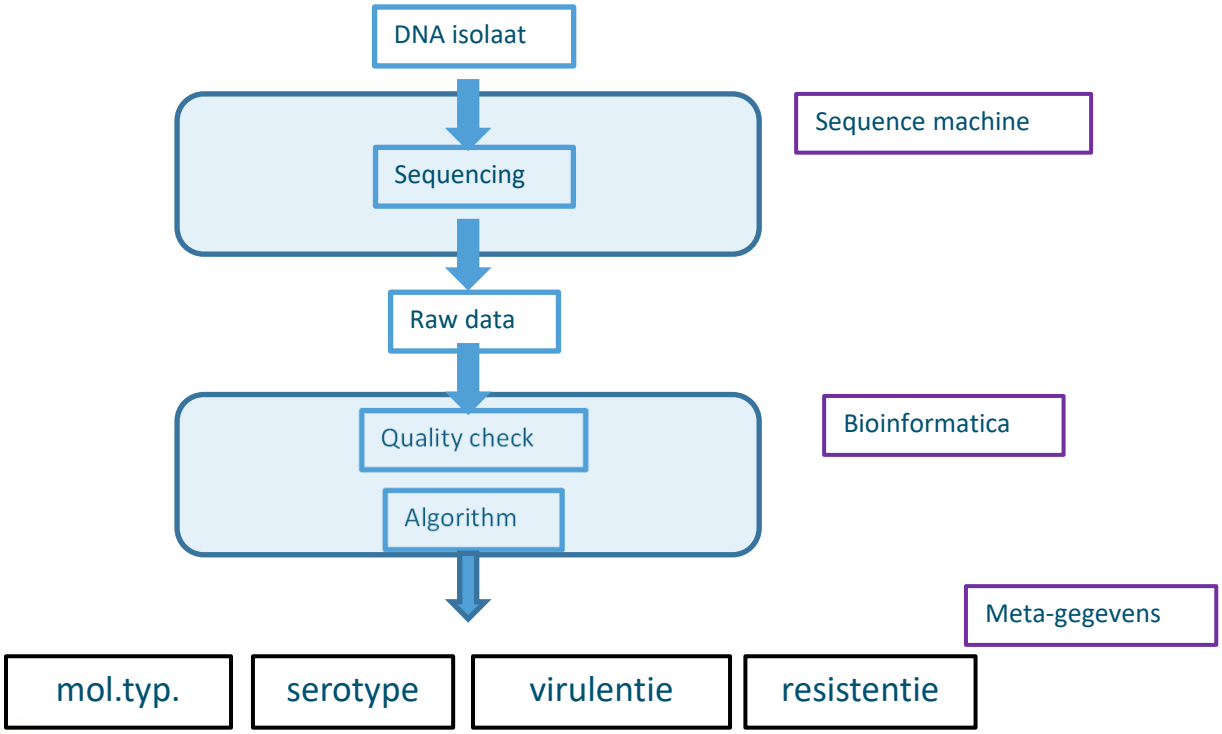
Bioinformatica Pipelines



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Moleculaire typering - Listeria

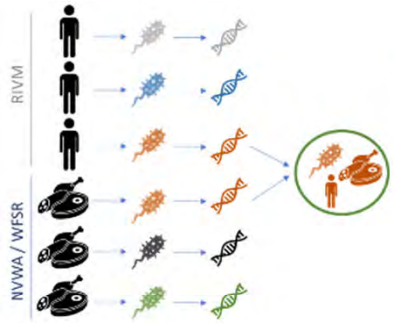
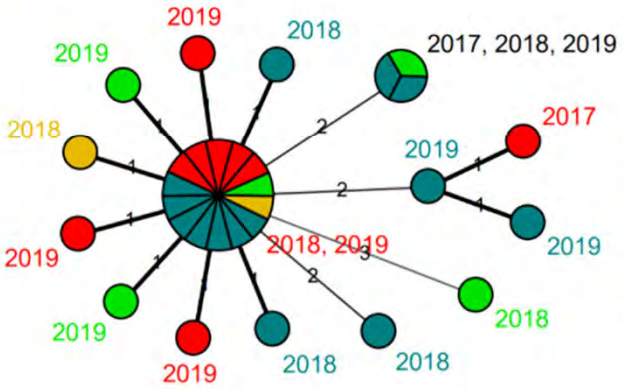
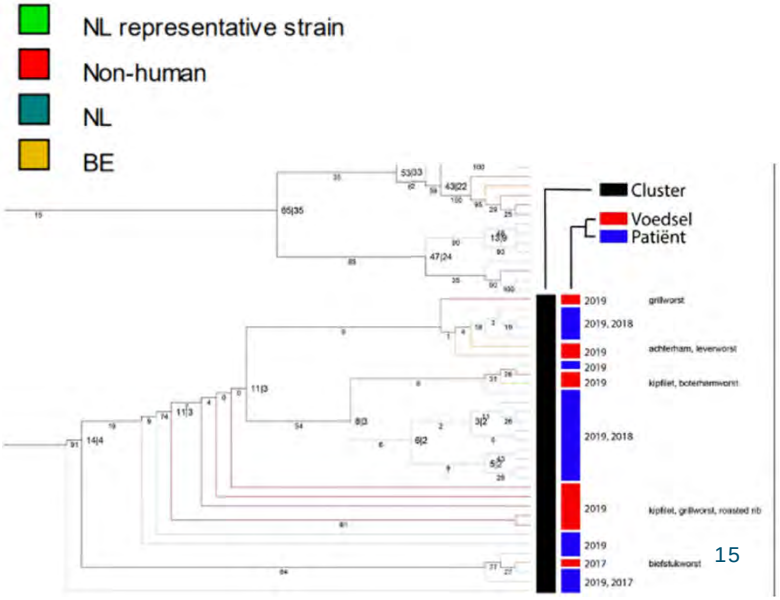


Figure 2. Minimum spanning tree (cgMLST, Institut Pasteur scheme) including sequences from 21 human *L. monocytogenes* isolates and nine non-human isolates from two countries, EU/EEA, 2017 to 2019





https://www.efsa.europa.eu/sites/default/files/scientific_output/PUBLIC_2019_ECDC-EFSA_ROA_UI-583_Listeria.pdf

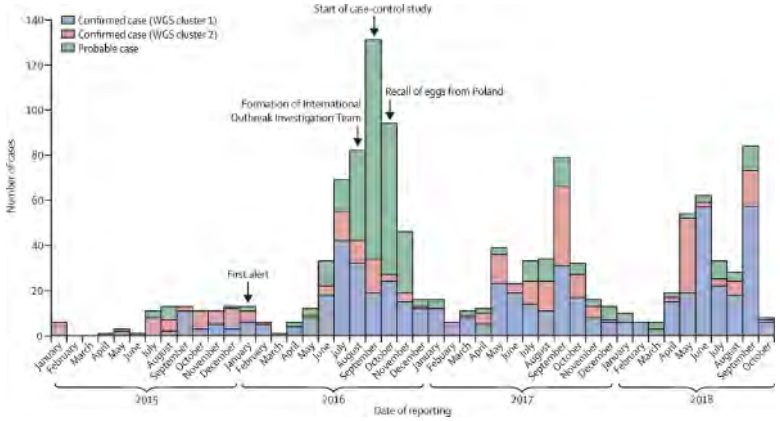
Samenvatting Listeria clusters (2015-2019)

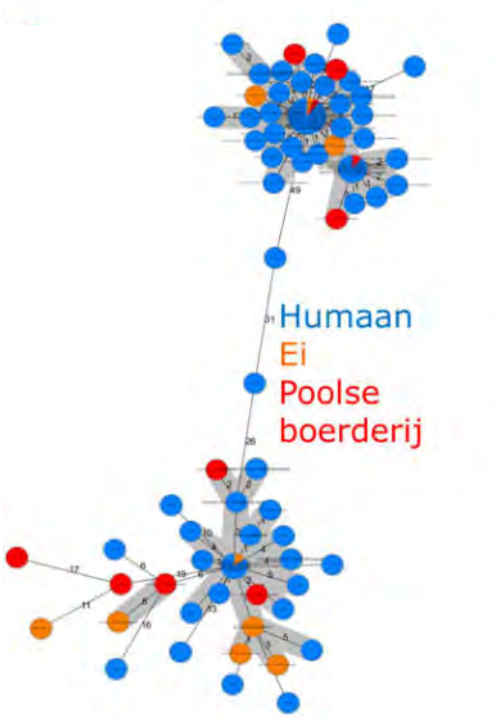
- 47 clusters *L. monocytogenes* isolaten (268 voedsel, 54 humaan)
- 36 clusters over meerdere jaren
- 31 clusters enkel voedsel isolaten, 16 clusters ook humane isolaten
- Waarsoorten:
  - 11 x rundvlees
  - 10 x pluimveevlees
  - 1 x varkensvlees
  - 3 x vleeswaren
  - 10 x zalm
  - 9 x forel, haring, garnalen

Prioriteit verwerken signalen:

1. Actieve humane uitbraken
2. Historisch humane uitbraken
3. Sporadische humane gevallen
4. Persistente stammen

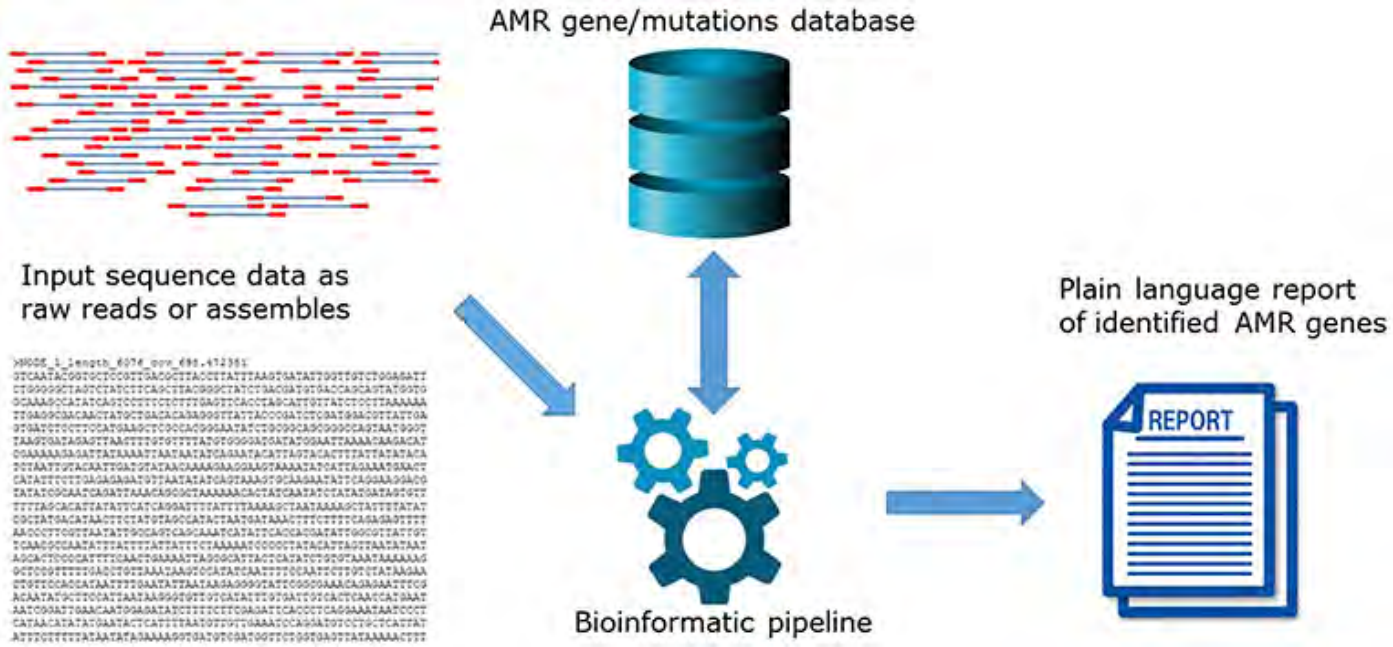
Moleculaire typering - Salmonella





https://www.sciencedirect.com/science/article/pii/S1473309919300477#fig3

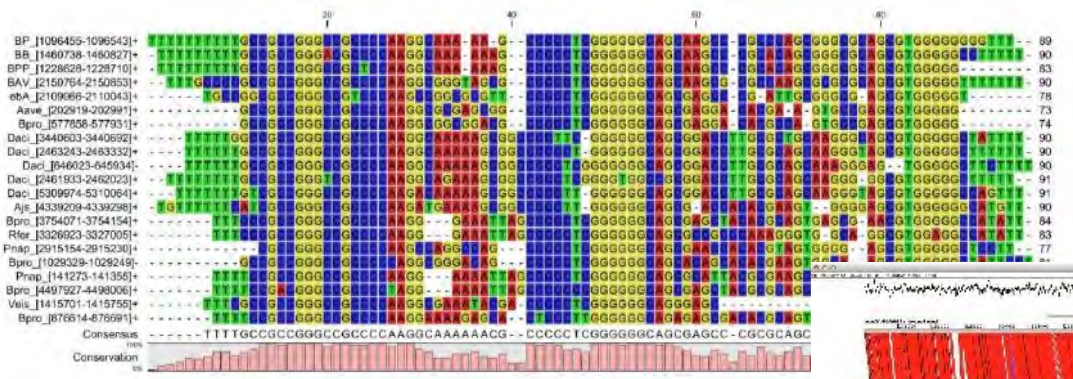
Resistentie, virulentie, mobiele elementen

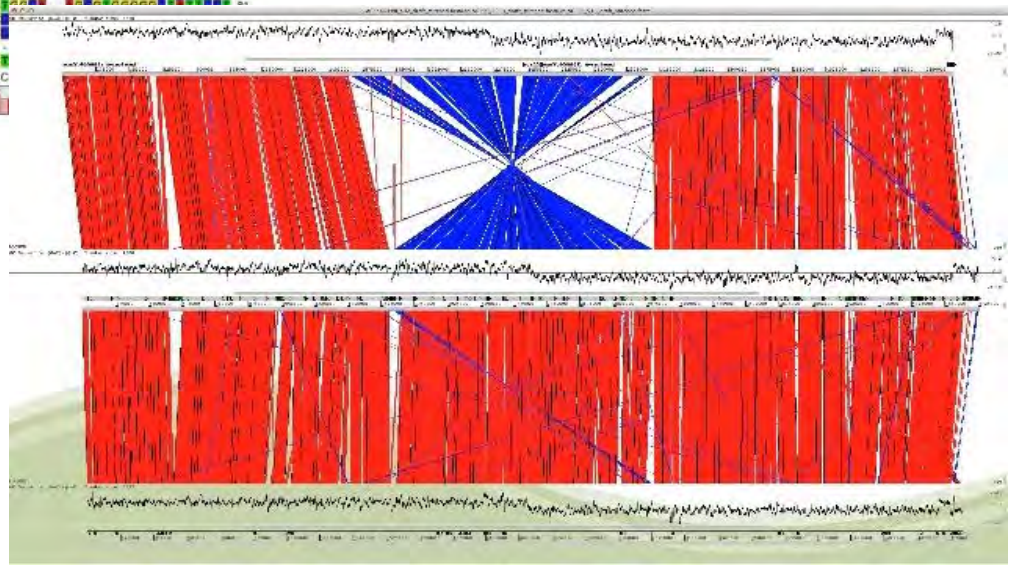


https://www.frontiersin.org/articles/10.3389/fpubh.2019.00242/full

EFSA plannen voor rapportages op basis van WGS

Comparative genomics





Virulentie Listeria

- inlA

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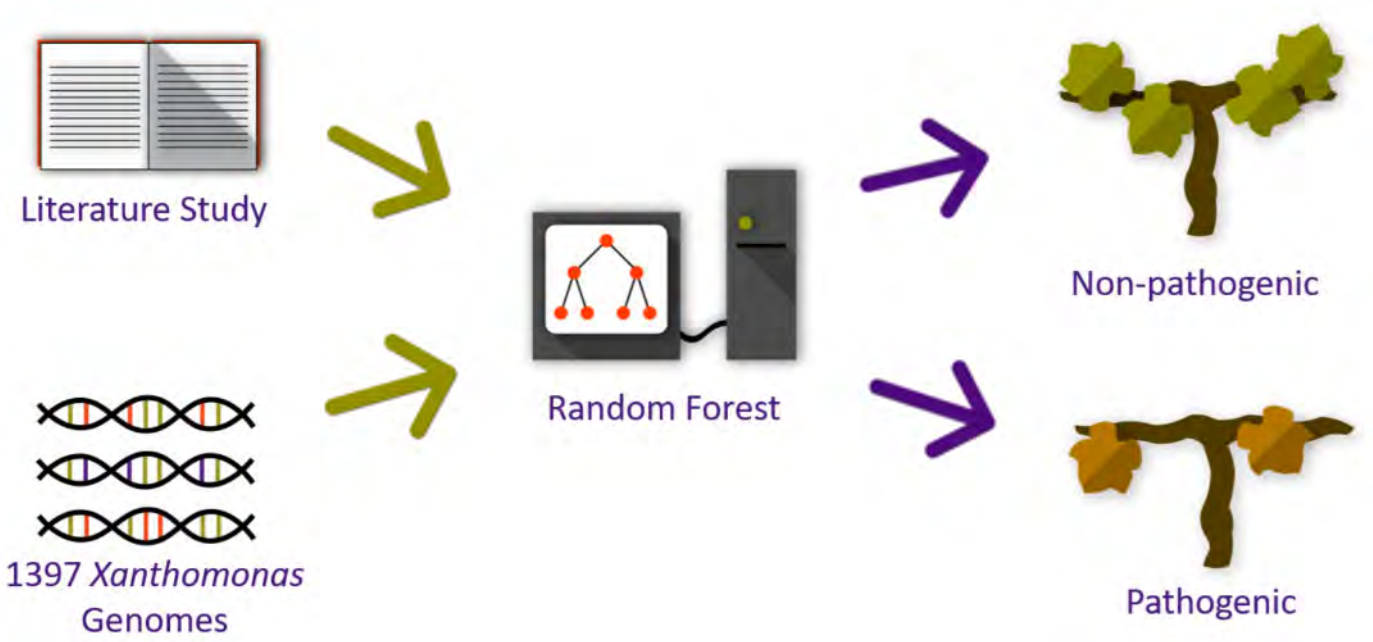
Machine learning



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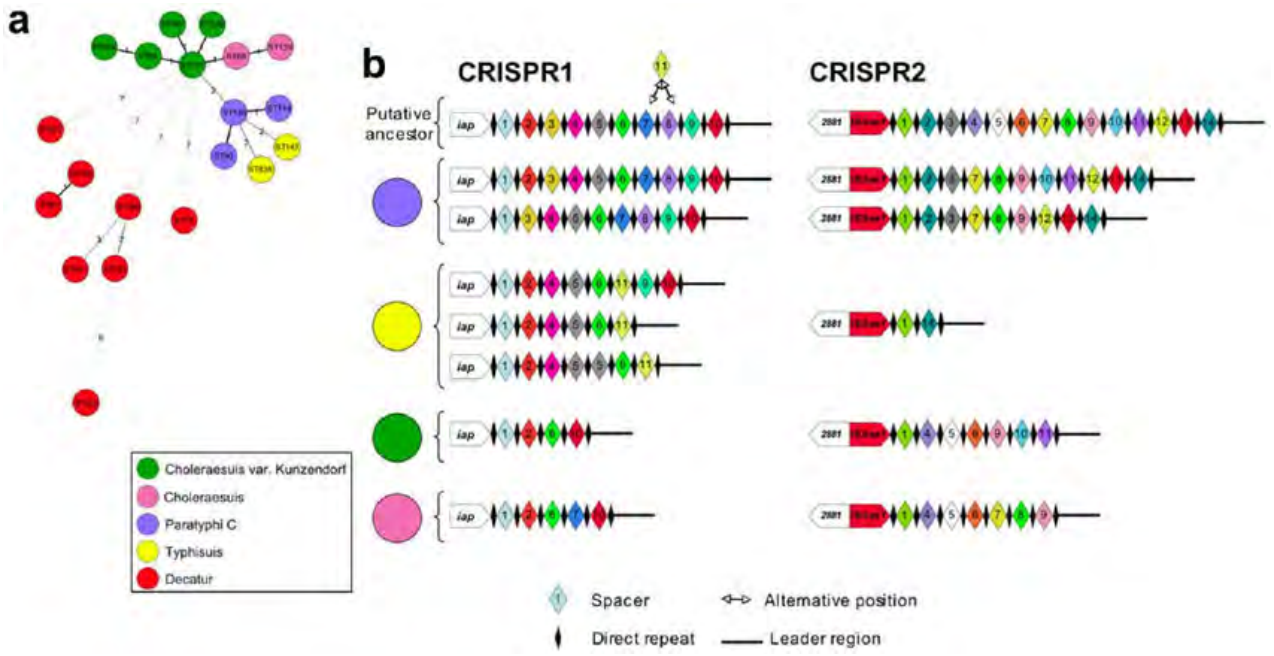
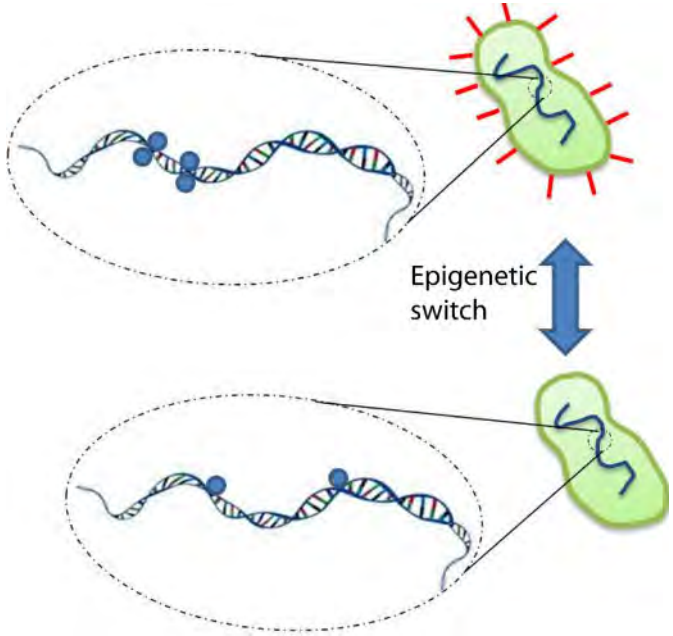
https://2019.igem.org/Team:Wageningen_UR/Results/Pathogenicity

Crispr typering



https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0036995.g002

Epigenetica



- Nanopore sequencing
- SMRT sequencing

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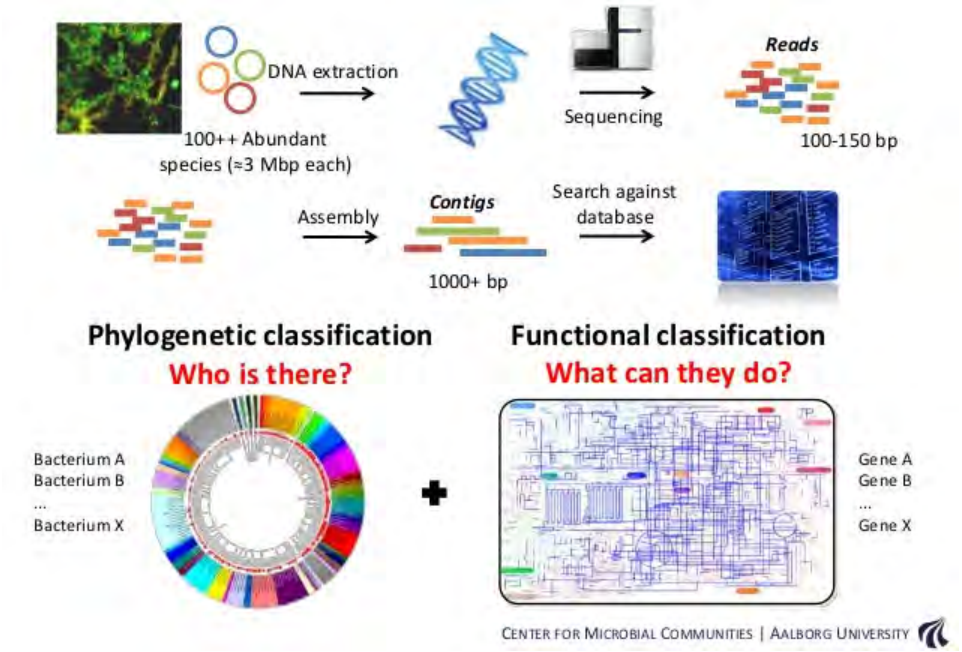
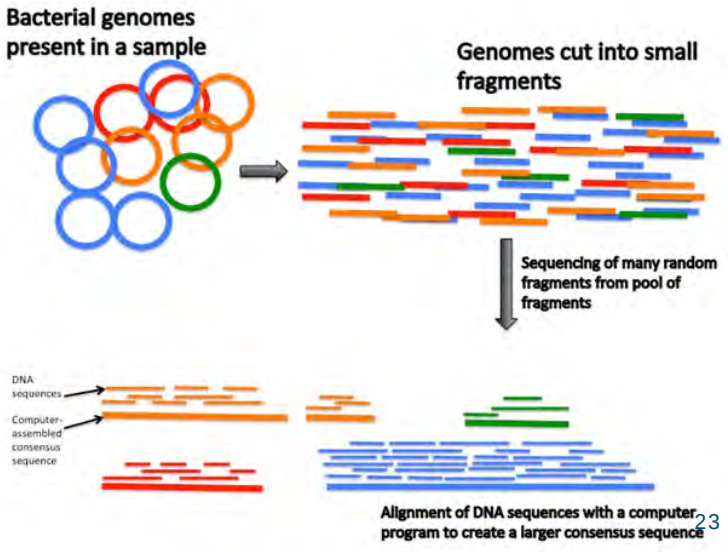
Metagenomics





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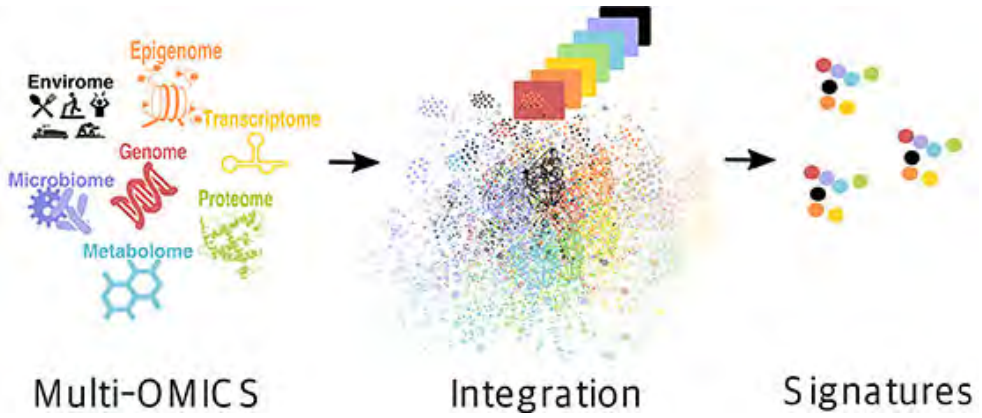
Omics combineren – multi-omics

- (Meta-)genomics – DNA
- (Meta-)transcriptomics – RNA
- (Meta-)proteomics - eiwit



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De potentie van Whole Genome Sequencing

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    - Wat kan deze bacterie?
        - Resistentie, virulentie, enzym productie, antibiotica productie