CA18105

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Risk-based meat inspection and integrated meat safety assurance

Genomic techniques in food safety diagnostics in comparison to classical culturing
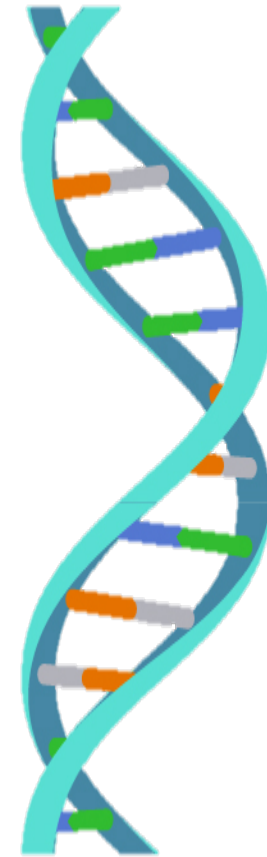
Martijn Bouwknegt

Research Manager Food Safety

Vion Food Group

Outline of this lecture

- Traditional methods used in the food industry

- Overview of genomics techniques

- Advantages and disadvantageous

- Examples used in Food Safety

  - WGS for resident *Salmonella*

  - Metagenomics for biofilm composition

- Discussion/conclusion

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Traditional detection methods

- Mandatory to test according to 2073/2005 (FSO, PHC)
  - Quantities
  - Presence/absence

- ISO-methods developed for bacterial culturing

- Well established, validated and standardized

- Very useful for their purpose
  - Specific
  - Detection of viable cells
  - Quantification (w.o. pre-enrichment step)

- Technological developments provide ample alternatives

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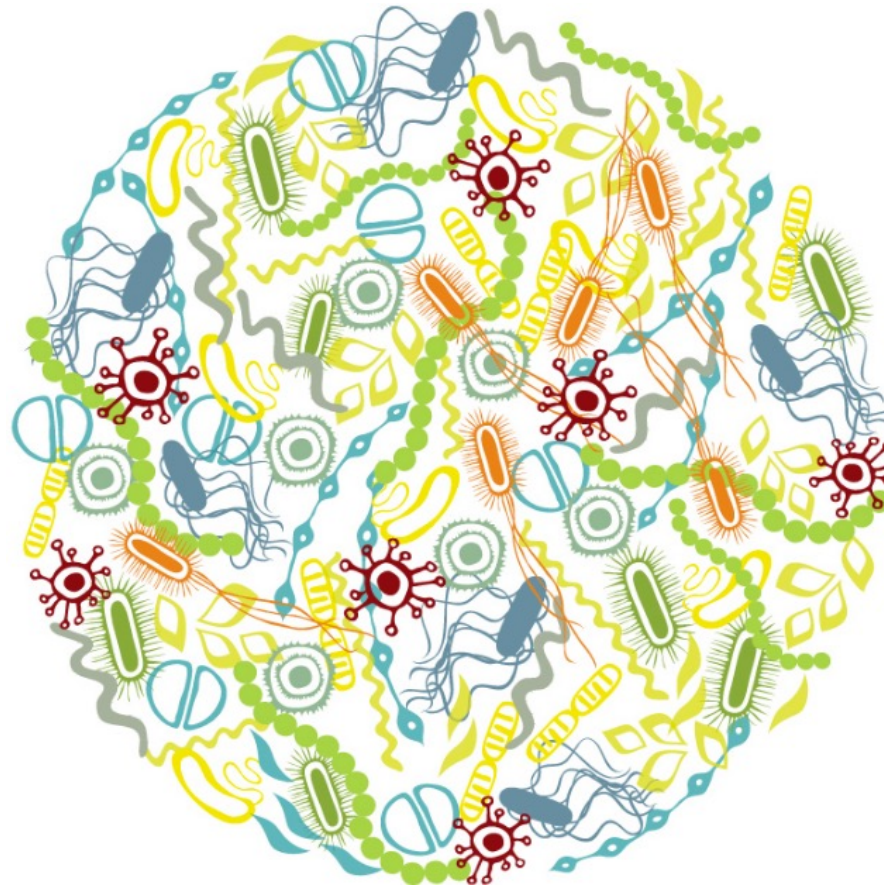# Genomic analysis: DNA or RNA-based diagnostics
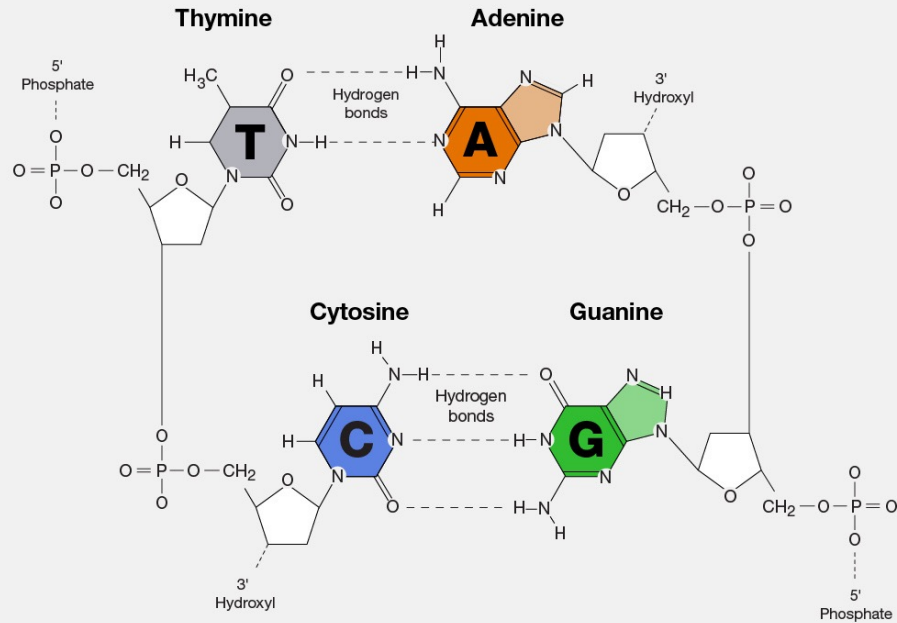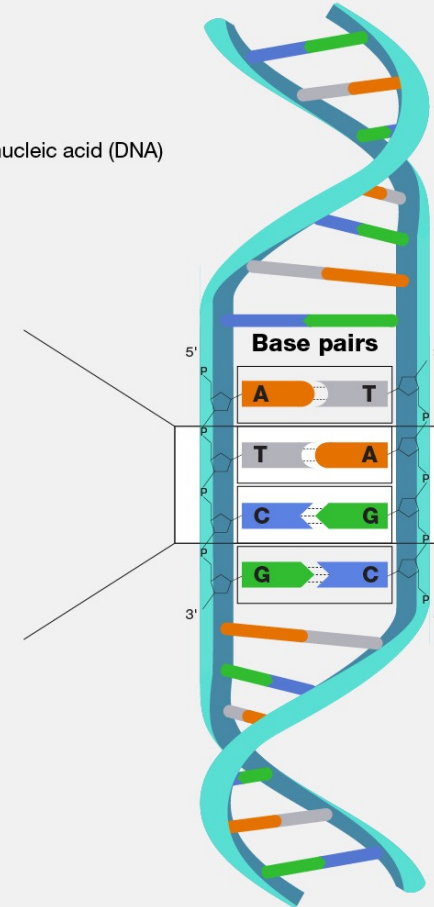
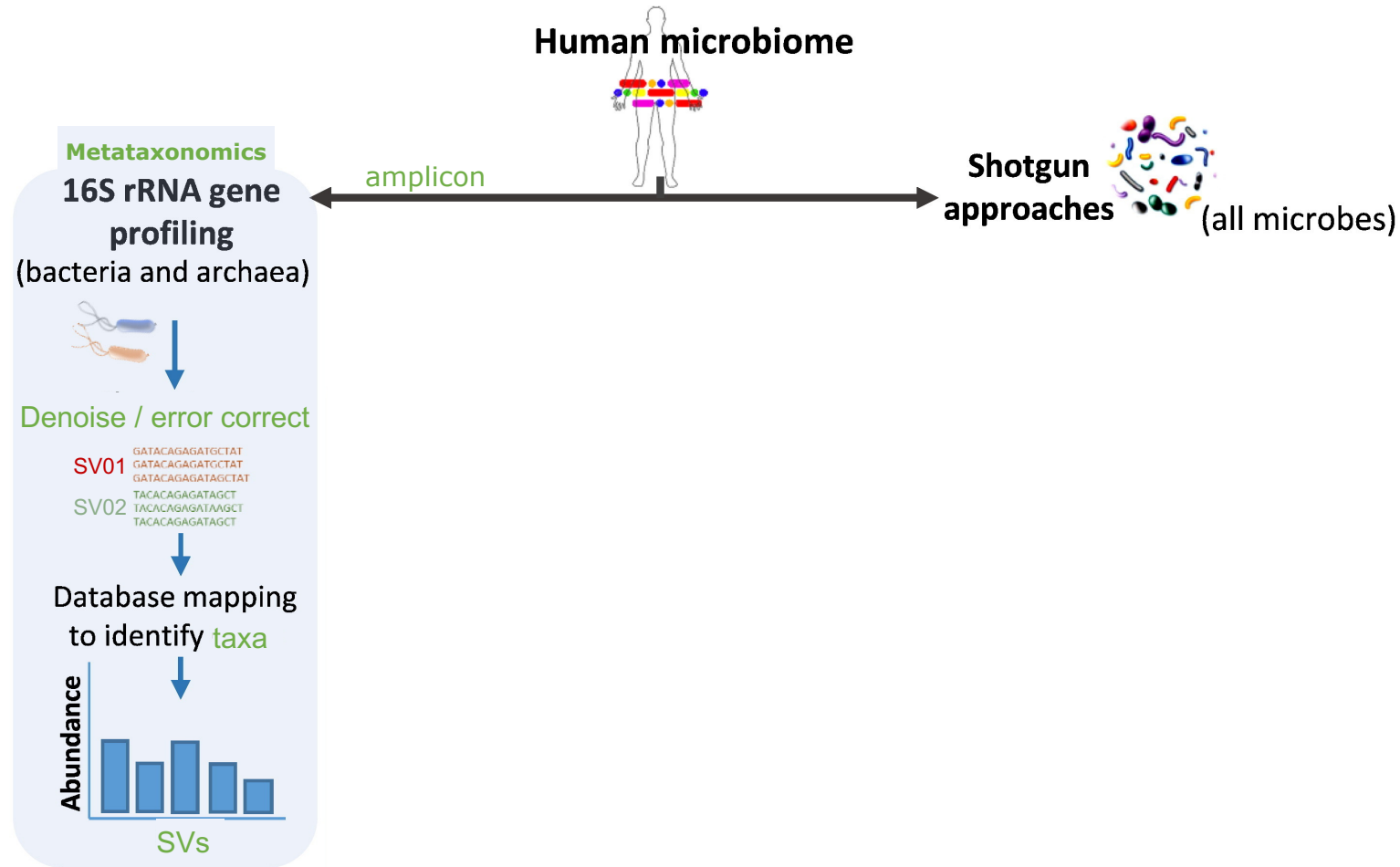# Genomics to unravel the microbiome

Microbiome

All available organisms in an environment or sample:
- Fungi
- Parasites
- Bacteria
- Viruses

>95% of microorganisms are not 'planktonic'

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Genomics to unravel the microbiome

Human microbiome

Metataxonomics
16S rRNA gene profiling
(bacteria and archaea)

amplicon

Shotgun approaches (all microbes)

Denoise / error correct

SV01 GATACAGAGATGCTAT
GATACAGAGATGCTAT
GATACAGAGATAGCTAT

SV02 TACACAGAGATAGCT
TACACAGAGATAAGCT
TACACAGAGATAGCT

Database mapping to identify taxa

Abundance

SVs

Genomic methods in summary

Culture

Whole genome sequencing

16S rRNA gene
PCR + NGS

Metagenome sequencing
(incl phages / fungi / DNA-viruses)
(RNA+DNA viruses :: VirCapSeq)

(Bacteria/Archaea/Fungi)

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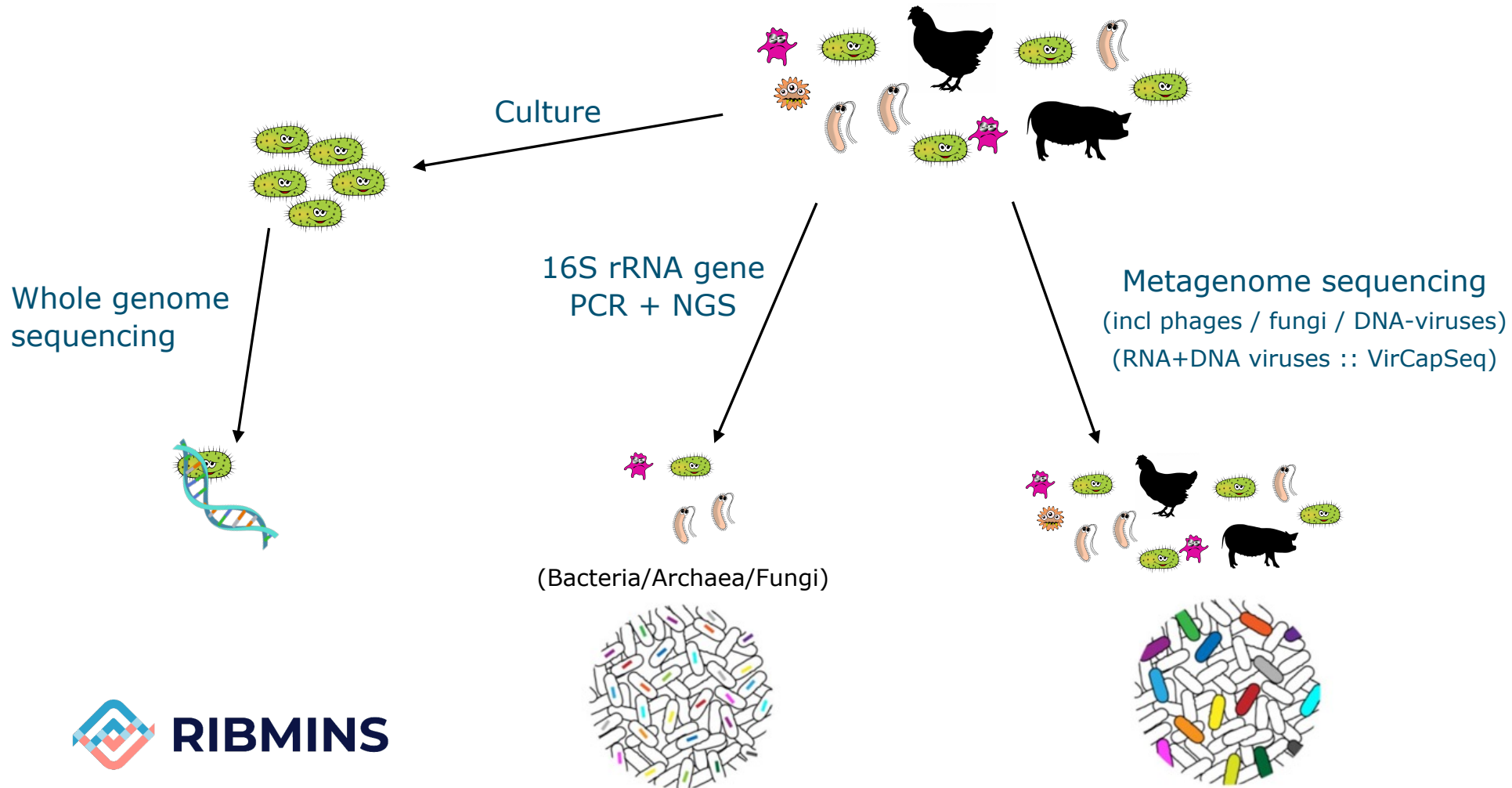
Puzzling with smaller DNA fragments
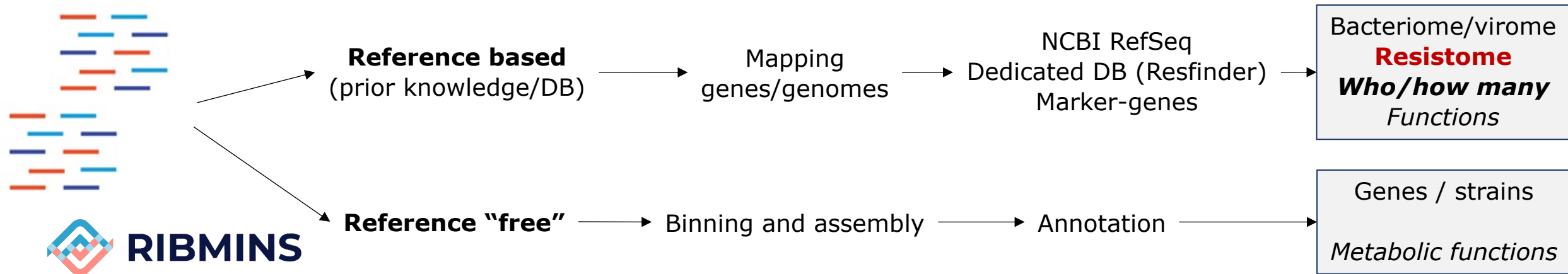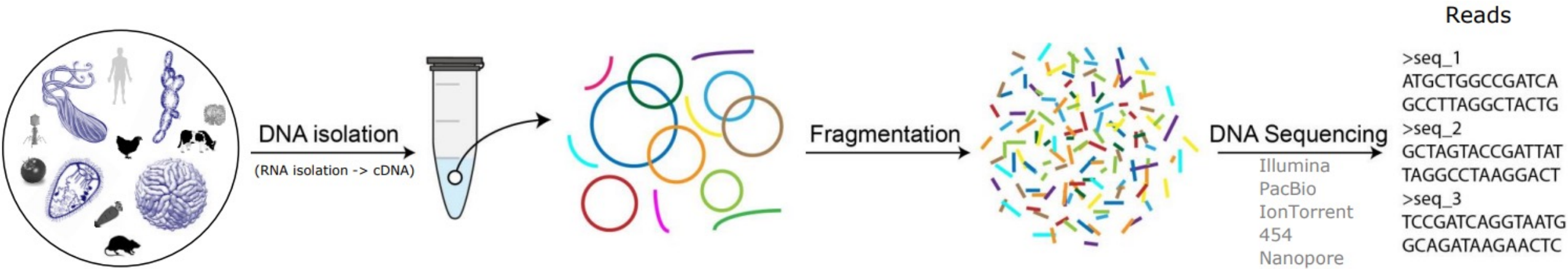
Genomic detection methods - advantages

- Multiple species detected in one analysis (~99% of microbial species uncultarable)

- More information through DNA sequences:
  - taxonomy
  - presence/absence of virulence genes; resistance genes
  - Source attribution

- Standardized format: enhances comparability

- Easy data sharing across companies and institutions

- Archive to examine later associations or occurrences

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Challenges in (meta)genomics

- Abundance of host DNA (e.g., pig DNA in pork)

- Distinction between living/dead (bacteria, molds, fungi) or intact/defective (viruses)

- Absolute quantification very challenging: count data not easily obtained

- Database dependent: lack of reference genomes or low-quality reference genomes

- Assembly: chimera formation (made-up sequences)

- Effect of DNA isolation methods

- Relatively expensive compared to culturing

- Data analysis requires specific training

Not feasible yet to replace traditional methods in routine monitoring

Knudsen *et al.* 2016, mSystems 1(5): e00095-16.

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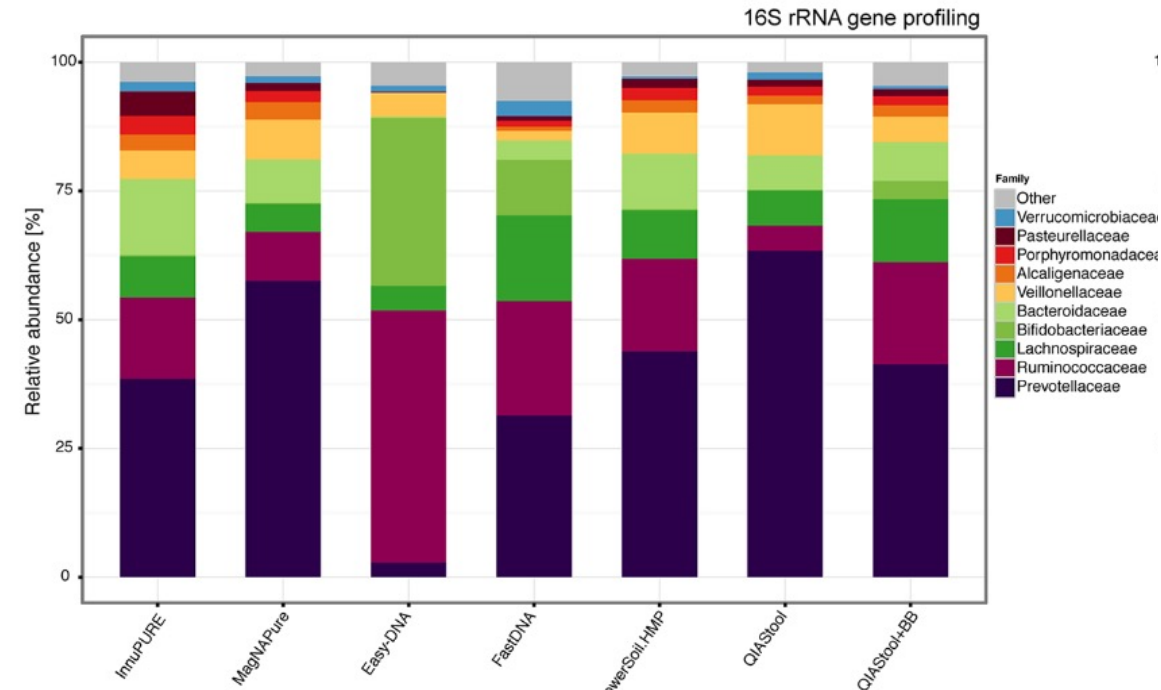
Case 1: Whole genome sequencing (WGS)

- Culture of a species → DNA isolation → full genome sequencing

- Especially useful for source tracking

- Estimated: ~65% of Salmonella contamination

  from in-house flora

- Can this be confirmed by sequencing?

Food Control 130 (2021) 108148

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Guidance document on the use of whole genome sequencing (WGS) for source tracking from a food industry perspective

Leen Baert [a,*], Peter McClure [b], Anett Winkler [c], James Karn [b], Martijn Bouwknegt [d], Adrianne Klijn [a]

[a] Nestlé Research, Société des Produits Nestlé S.A., Route du Jorat 57, 1000, Lausanne 26, Switzerland
[b] Mondelez International Global Food Safety RDQ, Linden 4, Bournville Lane, Birmingham, B30 2LU, United Kingdom
[c] Cargill Deutschland GmbH, Cerestarstr. 2, 47809, Krefeld, Germany
[d] Vion, Boseind 15, 5281 MA, Boxtel, the Netherlands

Meat Science 96 (2014) 1425–1431

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Quantifying the sources of Salmonella on dressed carcasses of pigs based on serovar distribution

J.H. Smid [a,b,*], A.H.A.M. van Hoek [b], H.J.M. Aarts [b], A.H. Havelaar [a,b], L. Heres [c], R. de Jonge [b], A. Pielaat [b]

[a] Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.178, 3508 TD Utrecht, The Netherlands
[b] National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control (CIb), P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[c] VION Fresh Meat West, Boseind 10, 5281 RM Boxtel, The Netherlands

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Approach

- Pig carcass samples are collected daily in all slaughterhouses in NL and DE

- Tested for ACC, entero's and *Salmonella* spp. in a commercial lab

- Salmonella isolates were stored at -20°C (n~100)

- Isolates from 2017 and 2018 were subjected to WGS at Wageningen University

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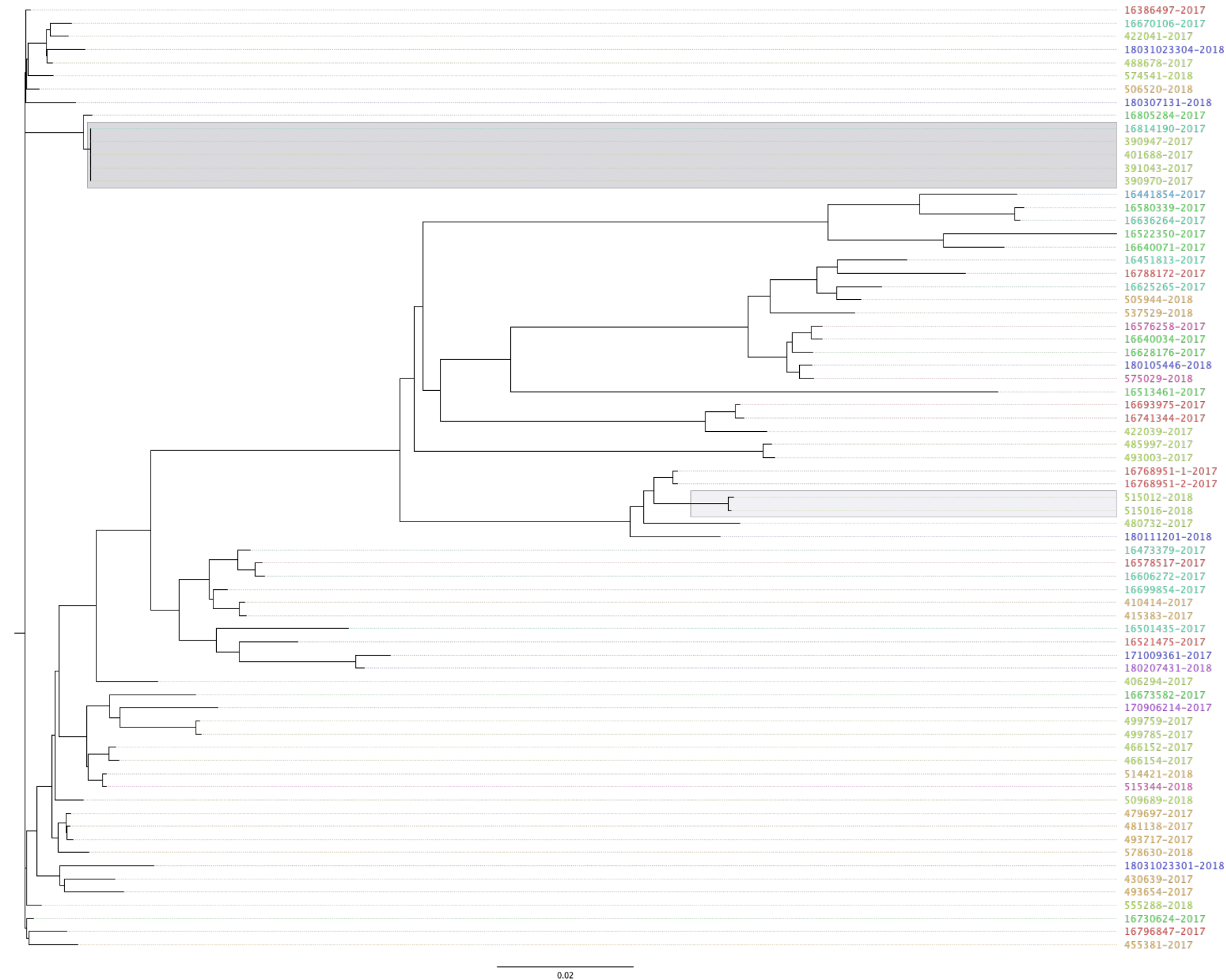
- Phylogenetic tree to show (dis)similarity

- Colors identify abattoirs in NL and DE

- Some clonal clusters present, but not majority

  Most clusters occur only for a short time period

Case 2: application of metagenomics for food safety

- Metagenomics can be used to answer three questions
  - What species are in a sample?
  - How many (relatively) of each of them are there?
  - What are they doing?
- >95% of bacteria are not 'planktonic', i.e., they reside in biofilms
- Our research interest:

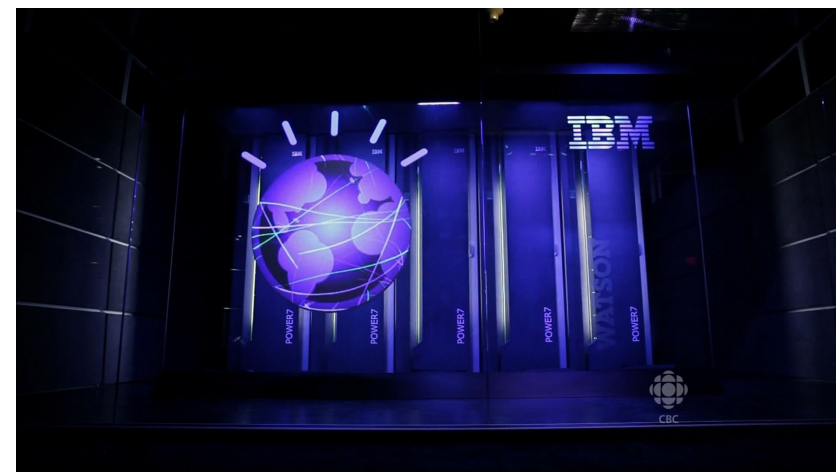 Can we use changes in biofilm composition in an abbatoir to predict *Salmonella* contamination events

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Changes in biofilm compositions?
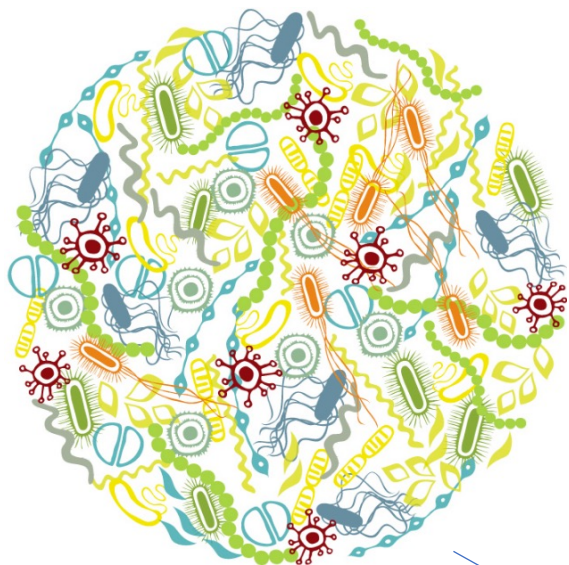
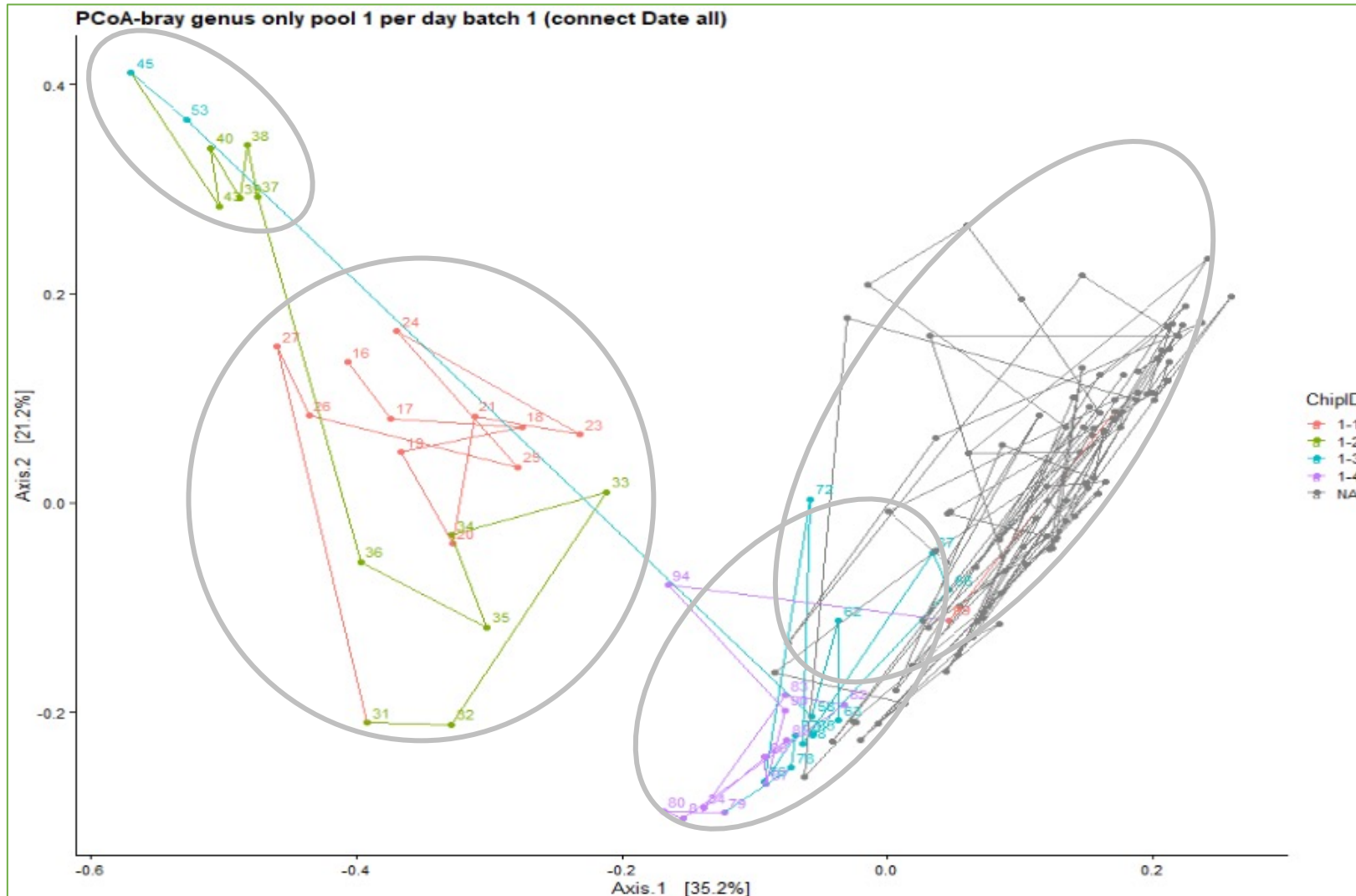
Biofilm signatures

Skin samples and data from abattoir

Data analyse en *machine learning*

RISK AHEAD

Early warning system?

1 year of skin sample collection from chilled carcasses (~4,000 samples)

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IBM ThermoFisher SCIENTIFIC Vion FOOD GROUP WAGENINGEN UNIVERSITY & RESEARCH

Clusters in sample composition that change over time

PCoA-bray genus only pool 1 per day batch 1 (connect Date all)

- Clusters follow a time pattern, suggesting jumps in microbiome composition
- Changes were not correlated with *Salmonella* events (power of analysis was low)
- Changes were not correlated with meta-data from the abattoir (temp, RH, line speed, cleaning regime)
- Unable yet to establish an early warning system

Conclusion

- Classical culture likely not replaced by genomics techniques in the short future
  - Detect living cells
  - Quantification
  - Issues in detection bias

- However, genomic techniques offer great possibilities for ad-hoc in-depth analysis
  - Source tracking
  - Source attribution
  - Microbiome analyses
  - Resistome questions

- Standardization of isolation, detection and data analysis techniques trivial for useable results

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More data does not automatically mean more answers!