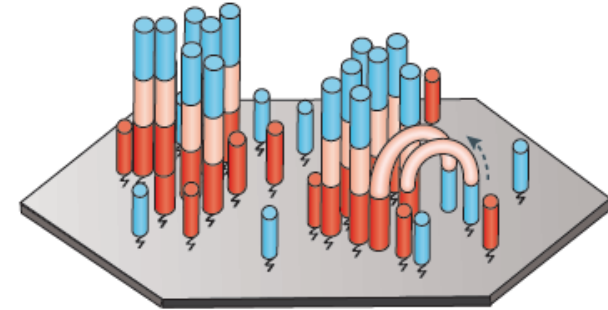


# Pathogenic organisms – no thanks: Use of next generation sequencing techniques in risk assessment and HACCP



Lisbeth Truelstrup Hansen

Professor

Microbial Food Safety and Environmental Hygiene

Mail: [litr@food.dtu.dk](mailto:litr@food.dtu.dk)

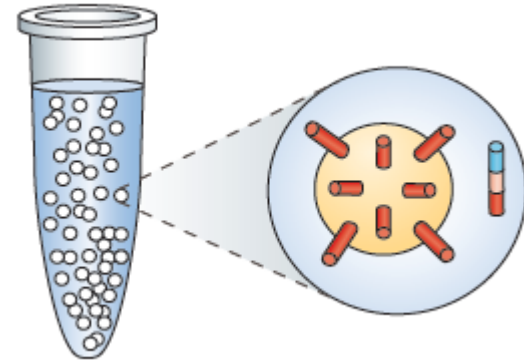
Telephone: 3588 6278

**DTU Food**

National Food Institute

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$$f(x+\Delta x) = \sum_{i=0}^{\infty} \frac{(\Delta x)^i}{i!} f^{(i)}(x)$$

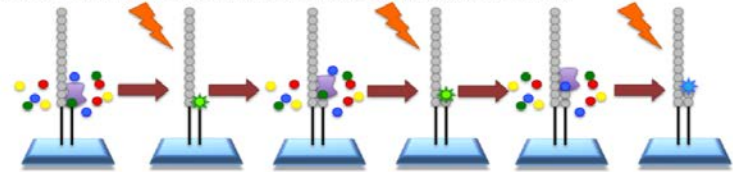


# Opportunities with NGS

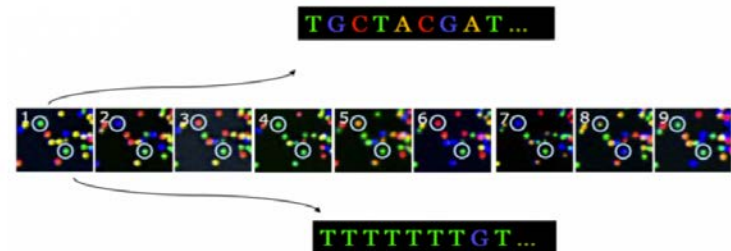
- Next generation sequencing methods (NGS) can be used to sequence large amounts of DNA or RNA sequences from **entire organisms** and microbial communities
- Many techniques including
  - Illumina (Solexa) sequencing
  - Roche 454 sequencing
  - Ion torrent: Proton/PGM seq.
  - SOLiD sequencing
  - PacBio
  - and so on

<https://www.ebi.ac.uk/training/online/course/ebi-next-generation-sequencing-practical-course/what-next-generation-dna-sequencing/illumina->

The process is repeated, adding one nucleotide at a time and imaging in between.



Computers are then used to detect the base at each site in each image and these are used to construct a sequence.

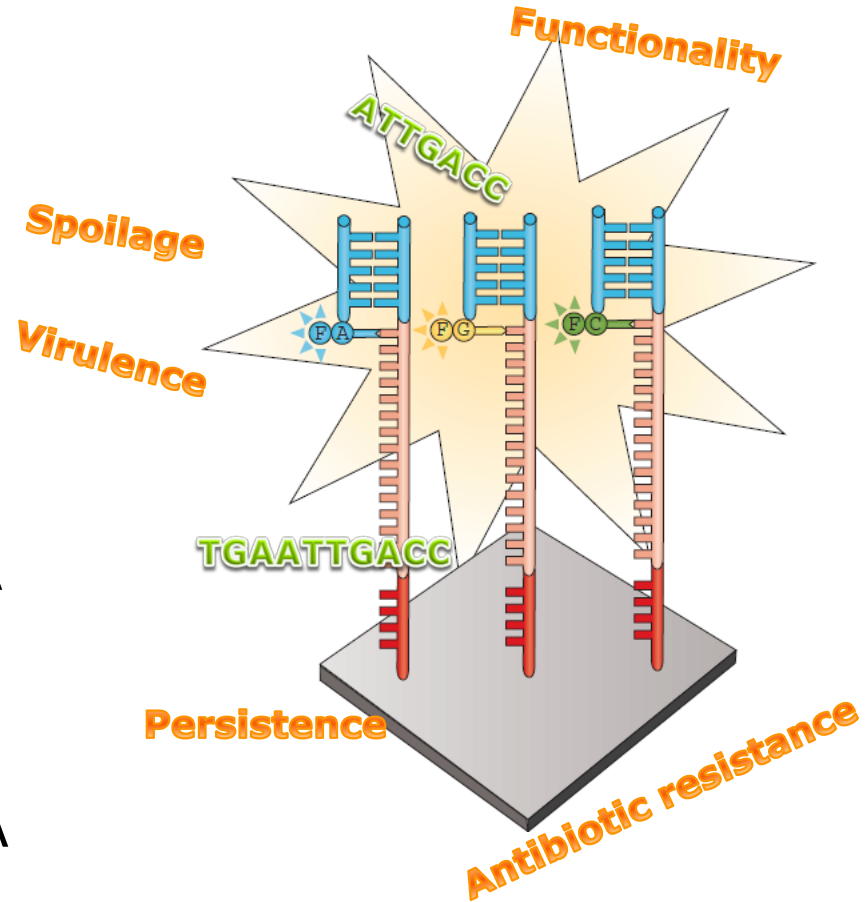


All of the sequence reads will be the same length, as the read length depends on the number of cycles carried out.

# Microbiology and NGS

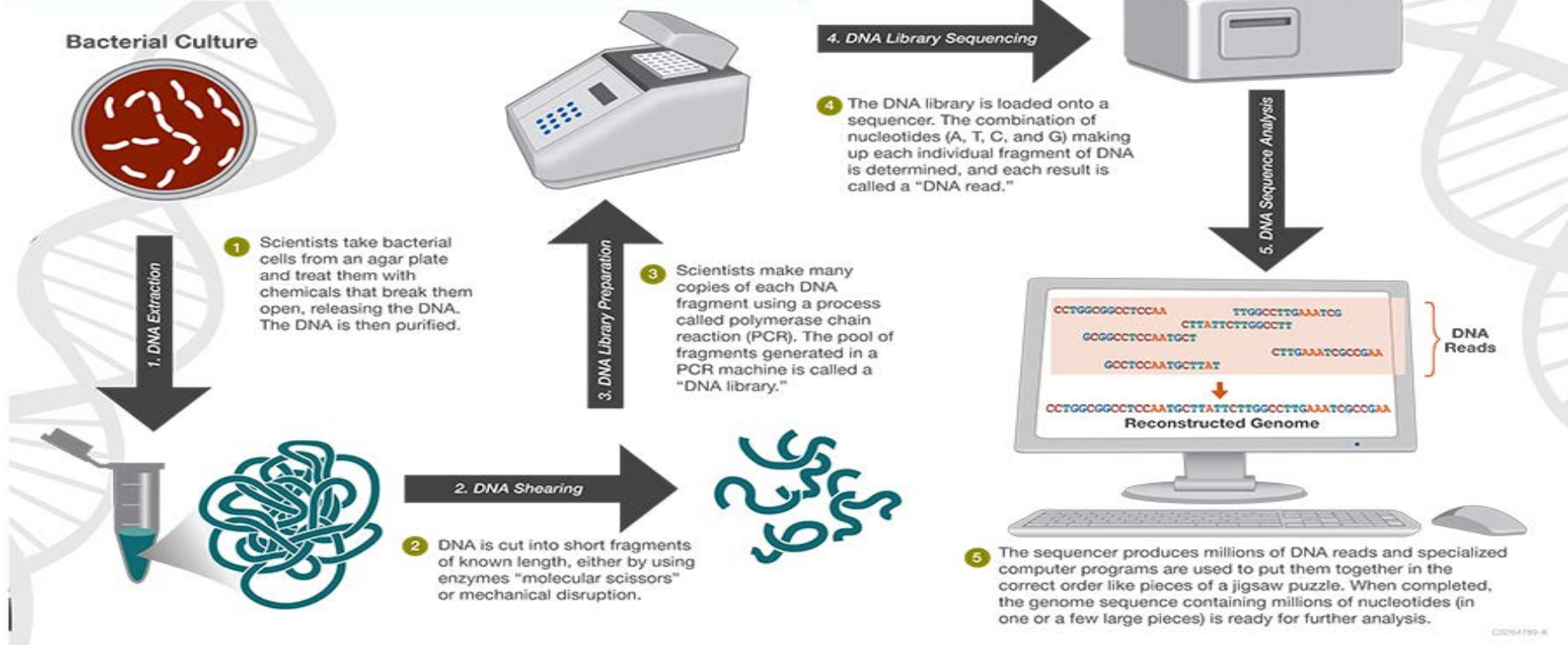
- NGS can help us to determine:
  - Who is there?
  - What are they doing?
  - Where do they come from?
  - What does it matter?

- Amplicon sequencing
  - Whole genome sequencing
  - Metagenomic sequencing
- DNA
- Transcriptomics (RNAseq)
    - Single organism
    - Entire community
- RNA

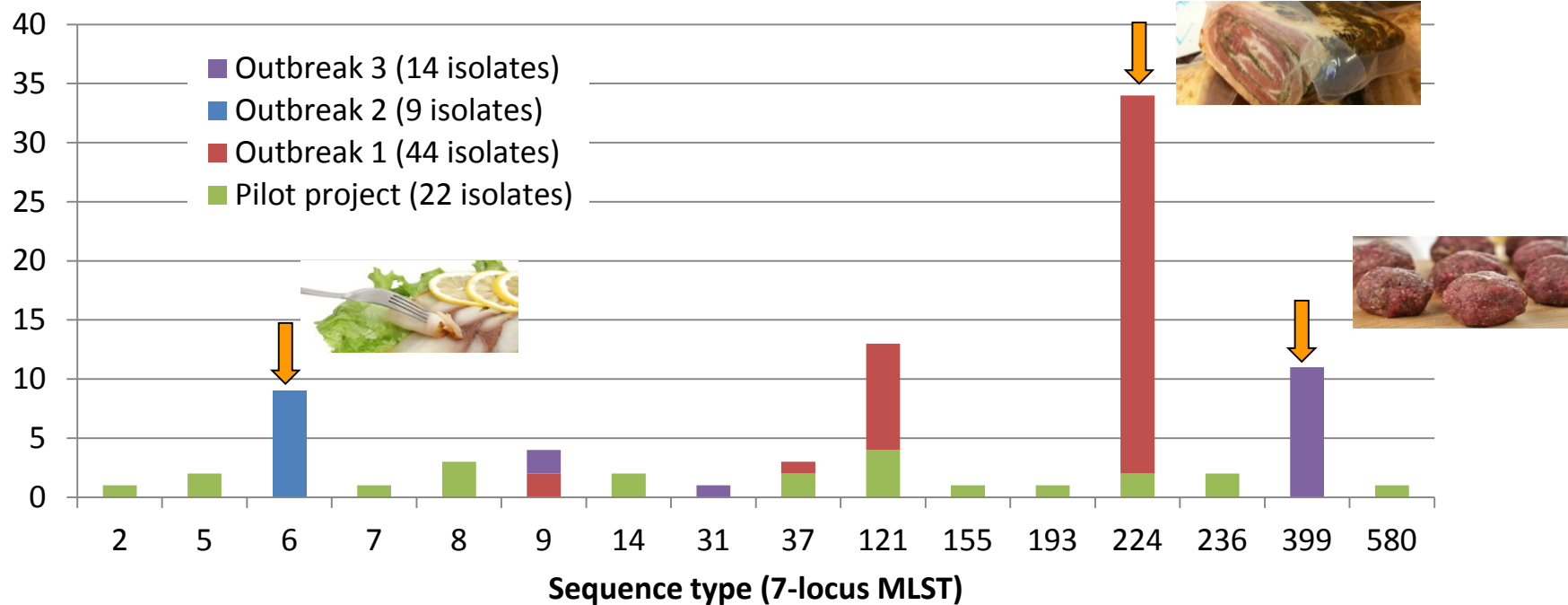


# The Whole Genome Sequencing (WGS) Process

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.



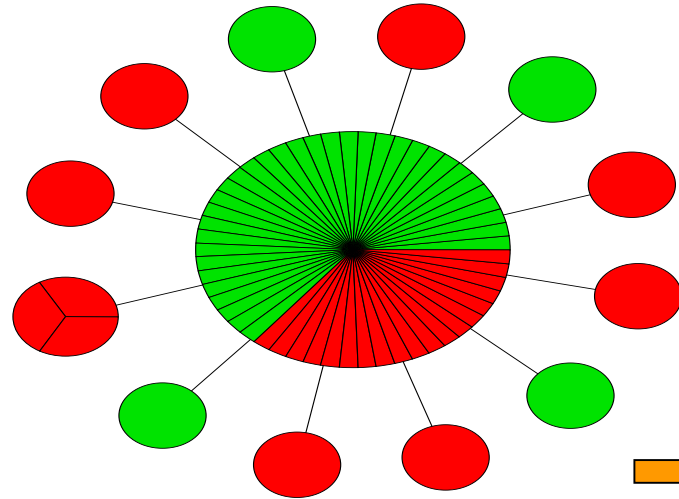
# Outbreak investigation: *Listeria monocytogenes* (SSI /FVST)



Match to human cases: WGS typing of isolates reason for finding link

Jensen AK, Nielsen EM, Björkman JT, Jensen T, Müller L, Persson S, Bjerager G, Perge A, Krause TG, Kiil K, Sørensen G, Andersen JK, Mølbak K, Ethelberg S Whole-genome sequencing used to investigate a nationwide outbreak of listeriosis caused by ready-to-eat delicatessen meat, Denmark, 2014. Clin Infect Dis.2016; 63(1), 64-70

# ST-224 outbreak summer 2014



- Isolates from 38 patients
- Isolates from 29 samples of food from producer A and several packaging and slicing companies



All cases and all food isolates (ST-224) linked to the outbreak had a max of 2 SNPs difference

—  
1 SNP



Jensen AK, Nielsen EM, Björkman JT, Jensen T, Müller L, Persson S, Bjerager G, Perge A, Krause TG, Kiil K, Sørensen G, Andersen JK, Mølbak K, Ethelberg S Whole-genome sequencing used to investigate a nationwide outbreak of listeriosis caused by ready-to-eat delicatessen meat, Denmark, 2014. Clin Infect Dis.2016; 63(1), 64-70

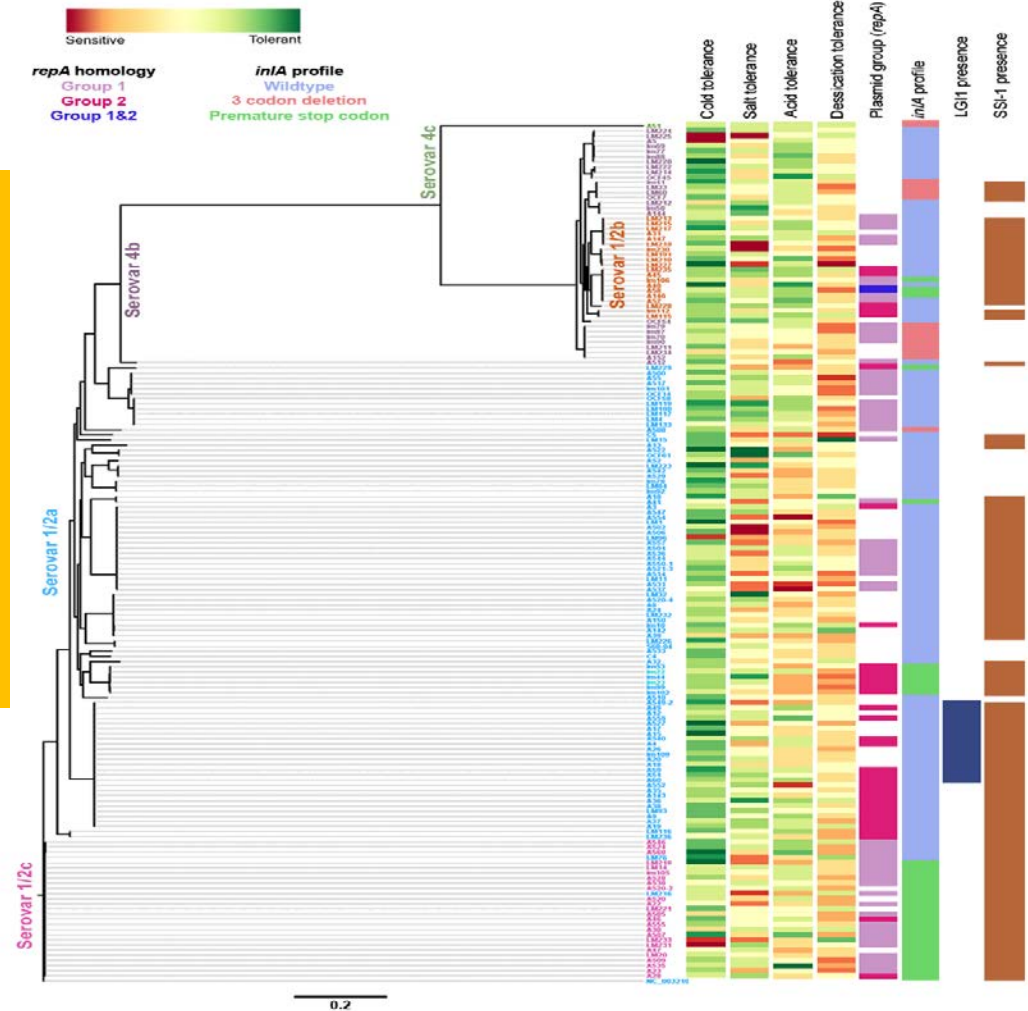
# WGS of 166 *L. monocytogenes*

- Strains also characterized for growth at 4°C, 6% NaCl, pH 5 and desiccation tolerance
- Tolerance was associated with full *inlA* gene and presence of plasmid
- New markers for higher fitness and potential for survival?

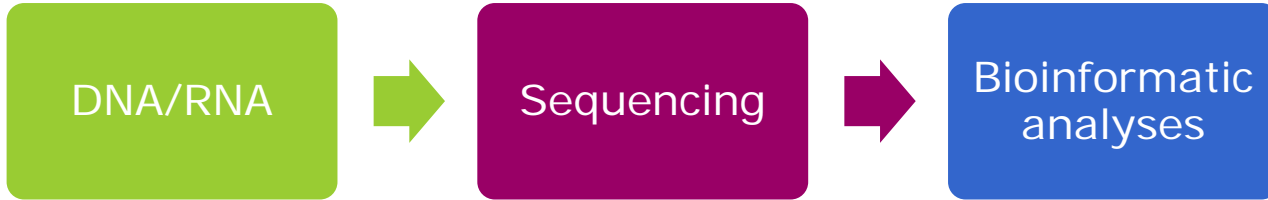
ORIGINAL RESEARCH ARTICLE  
Front. Microbiol. | doi: 10.3389/fmicb.2017.00369

## Genotypes Associated with *Listeria monocytogenes* Isolates Displaying Impaired or Enhanced Tolerances to Cold, Salt, Acid, or Desiccation stress

Patricia Hingston<sup>1</sup>, Jessica Chen<sup>1</sup>, Bhavjinder K. Dhillon<sup>2</sup>, Chad Laing<sup>1</sup>, Claire Bertelli<sup>1</sup>, Victor Gannon<sup>1</sup>, Taurai Tasara<sup>1</sup>, Kevin Allen<sup>1</sup>, Fiona S. Brinkman<sup>1</sup>, Lisbeth Truelstrup Hansen<sup>3</sup> and Siyun Wang<sup>1\*</sup>



# Metagenomic

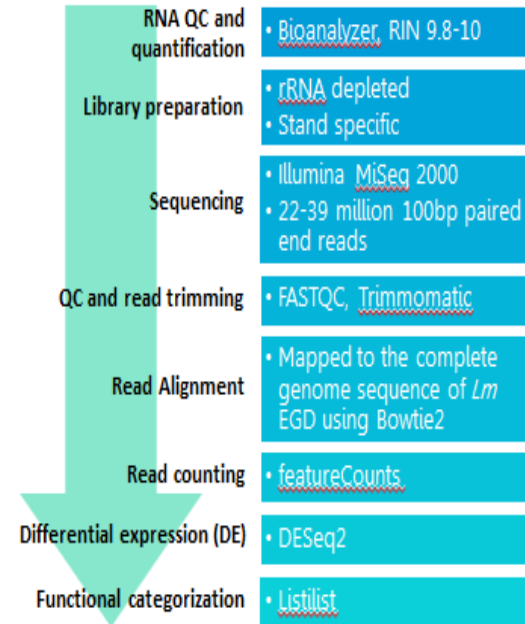


- Culture independent – shot gun sequencing

## • What can we find?

- Community composition
- Pathogens (all, viruses, parasites and bacteria)
- Metabolism
- Virulence
- Antibiotic resistance genes
- Genes and/or expression

### RNA seq workflow



Hingston et al. 2017 In prep.



# Transcriptome

- Genes expressed in *L. monocytogenes* growing at 4°C in different growth phases – markers of activity?

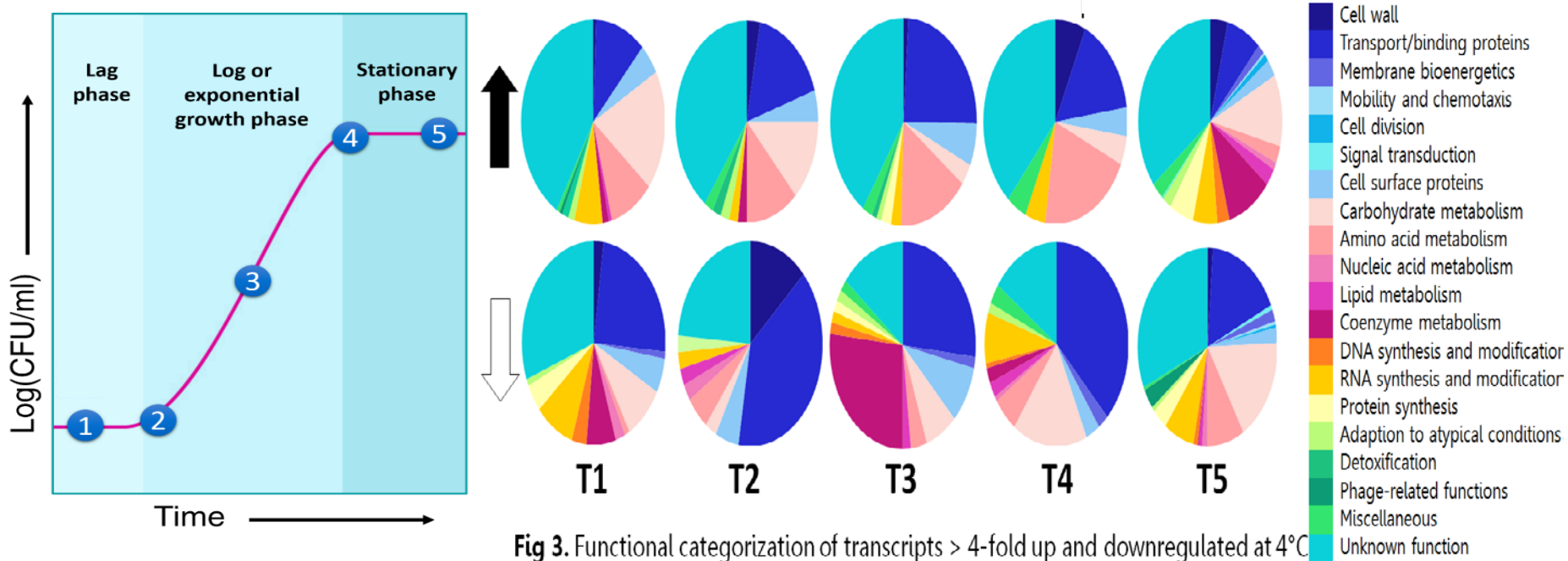
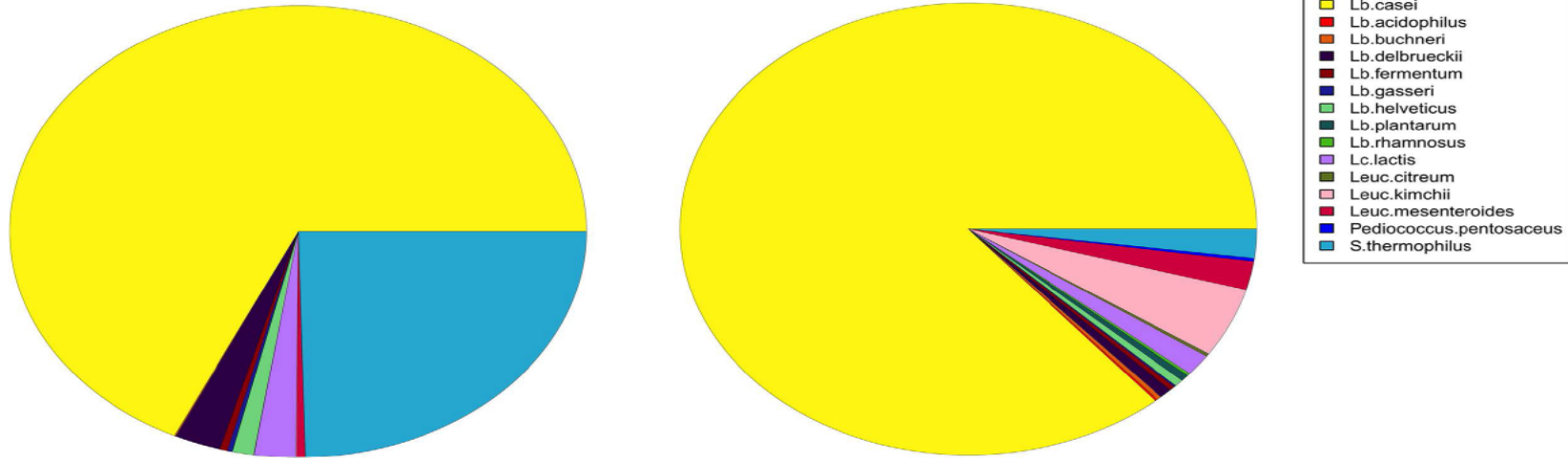


Fig 3. Functional categorization of transcripts > 4-fold up and downregulated at 4°C

# Metagenomic – characterize cheese ripening

Normal temperature

High temperature

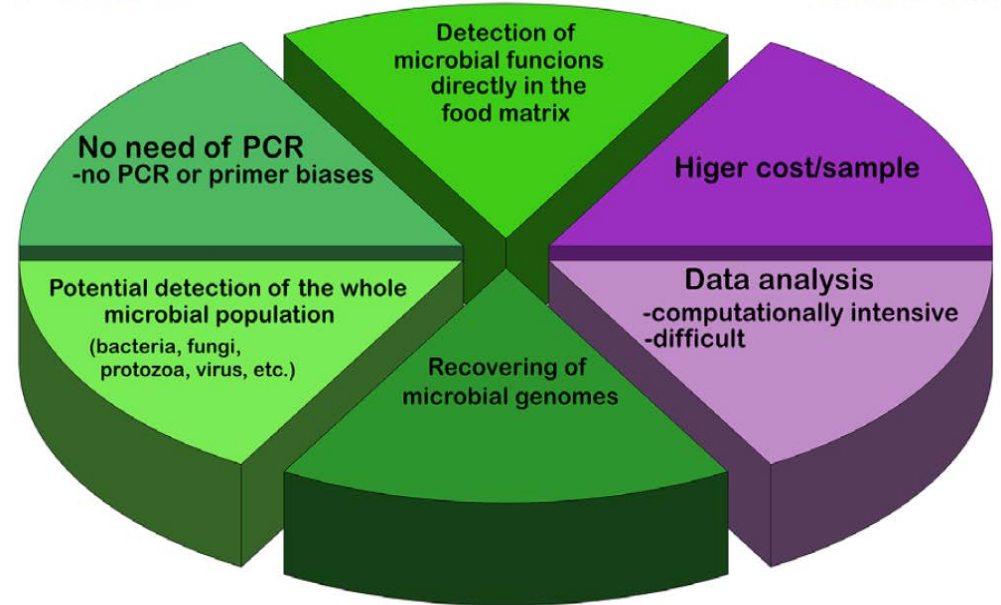


**Figure 4. Higher temperature boosts NSLAB amino acid metabolism.** Taxonomic assignment of the genes belonging to KEGG amino acid metabolism in the samples of cheese core from the second experiment, at 30 days of ripening. Only species belonging to Firmicutes are reported. A, ripening at standard conditions; B, higher temperature.

# Limitations and opportunities

- Culture/culture independent
- Cost
- Technical expertise
- Limit of detection
- Mapping of sequences
- Nucleic acid extraction from food samples, e.g., milk powder
- Privacy of data?
- Comparability of data?
- Software options

## PROS



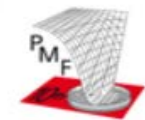
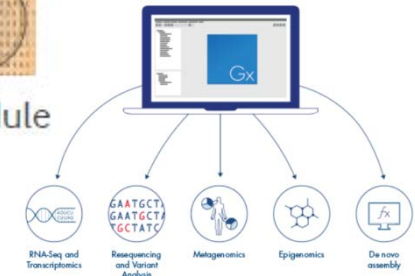
**Metagenomics** (De Filippis et al. 2017. Microb. Biotech.)

# Summary and Perspectives

- NGS can currently be used to conduct microbial source tracking and observe shifts in populations and metabolism
- Routine usage to assess microbial risks and verification of our HACCP plans?
  - Integrating NGS in food safety and quality a challenge
  - Topics at international conferences 2017
- Need for user friendly software and big data management
  - Also, don't use a steamroller to crack a nut

## Center for Genomic Epidemiology

Genome analysis module



CÓRDOBA, SEPTEMBER, 2017  
**ICPMPF10**  
 INTERNATIONAL CONFERENCE ON PREDICTIVE MODELLING IN FOOD