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IBERO-AMERICAN CONGRESS ON BIOTECHNOLOGY
7-9 APRIL 2022

FCT Fundação para a Ciência e a Tecnologia

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Seafood Age

Dr. Alejandro Garrido-Maestu

Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

Braga, Portugal

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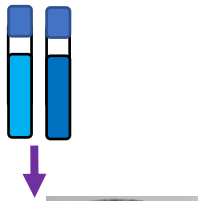
Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

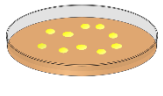
Classical

24h

24h



24h



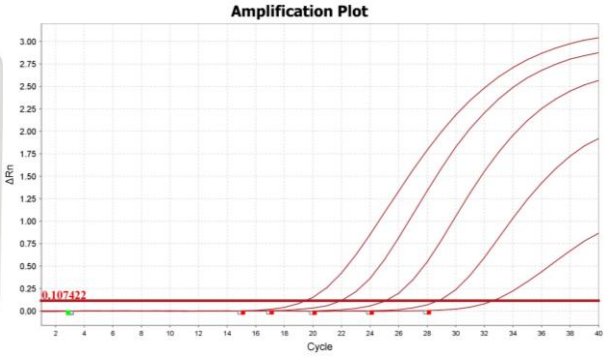
24h

24h

Molecular

24h

DNA extraction



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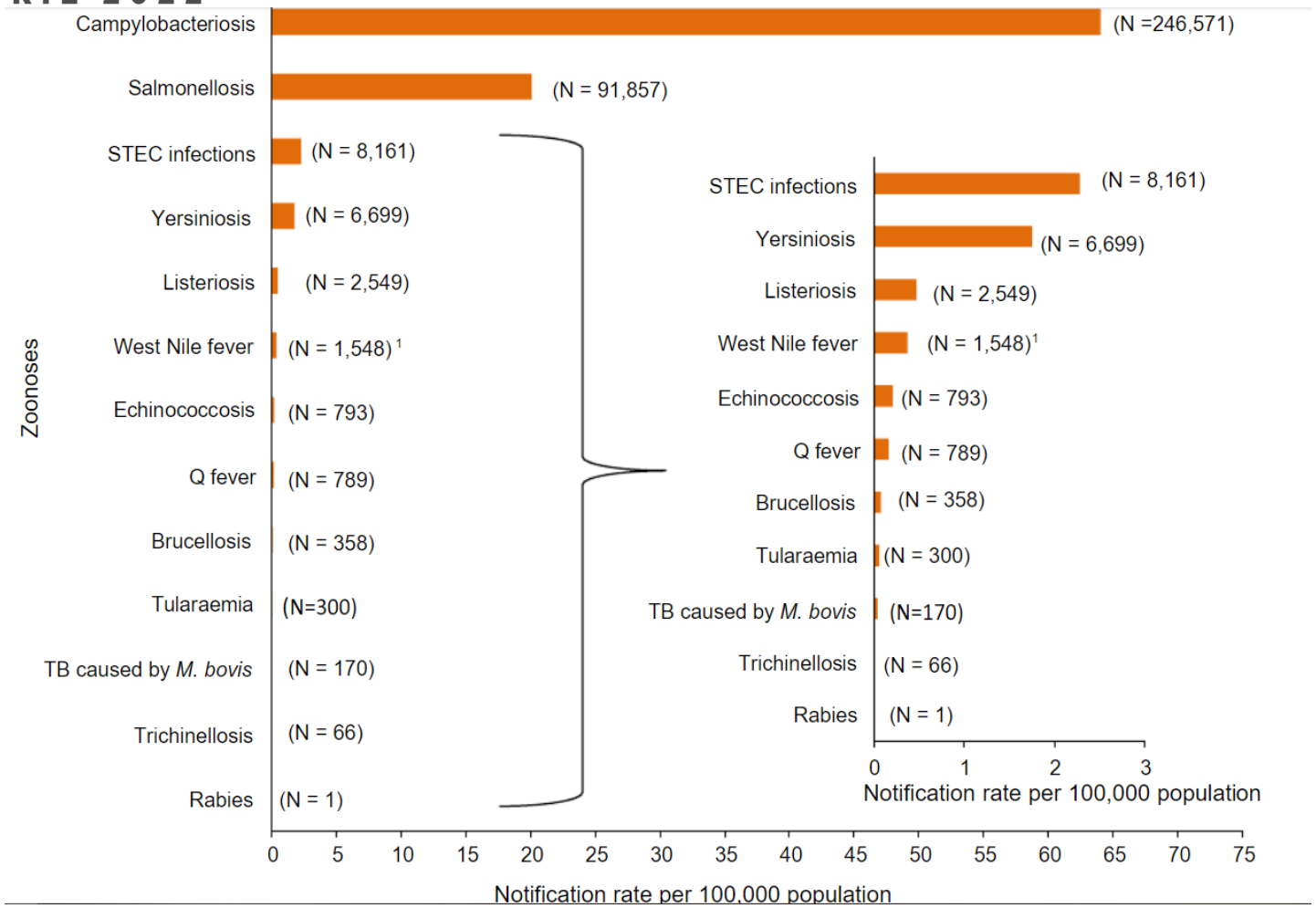
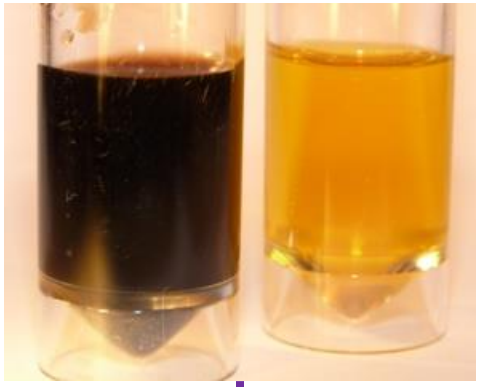


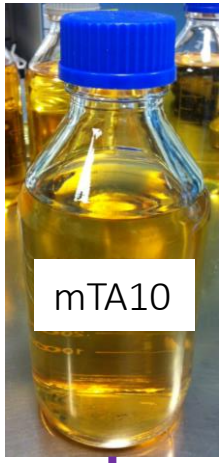
https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5926

Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

Multiplex assays

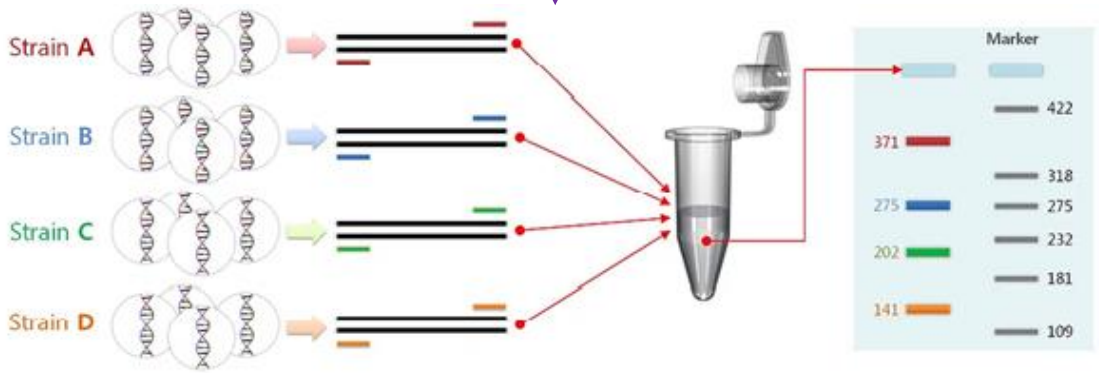
Culture

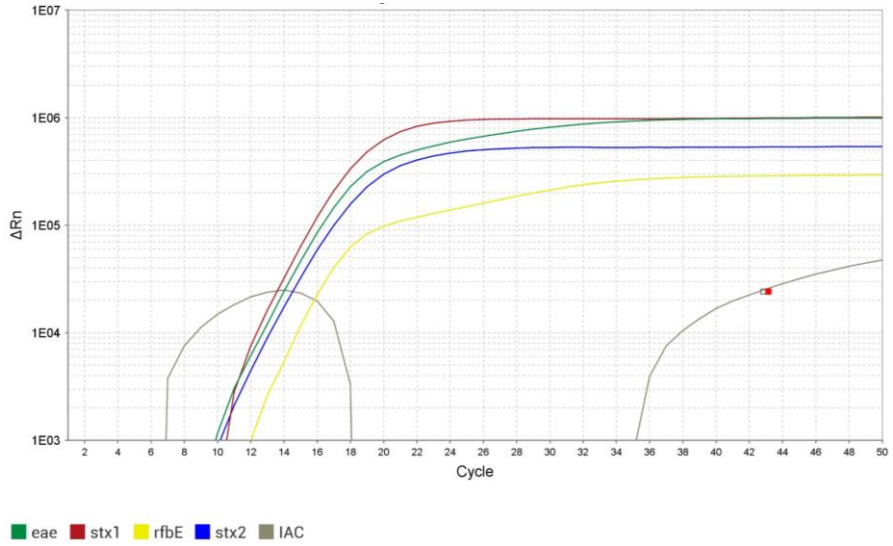


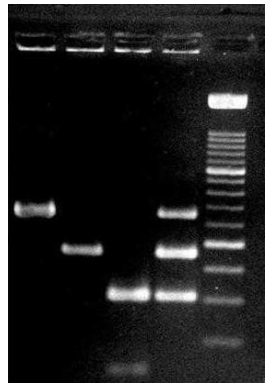


DNA extraction

Analysis



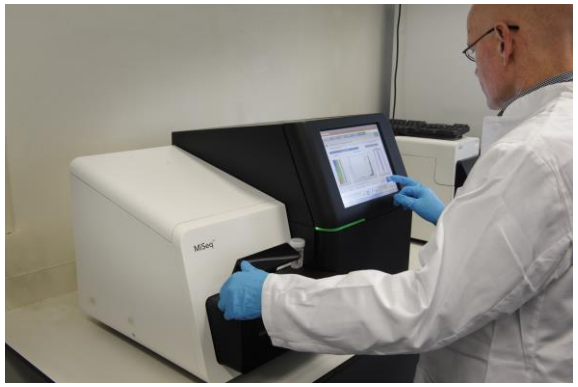




Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

Sequencing platform

Illumina



PacBio

Oxford Nanopore

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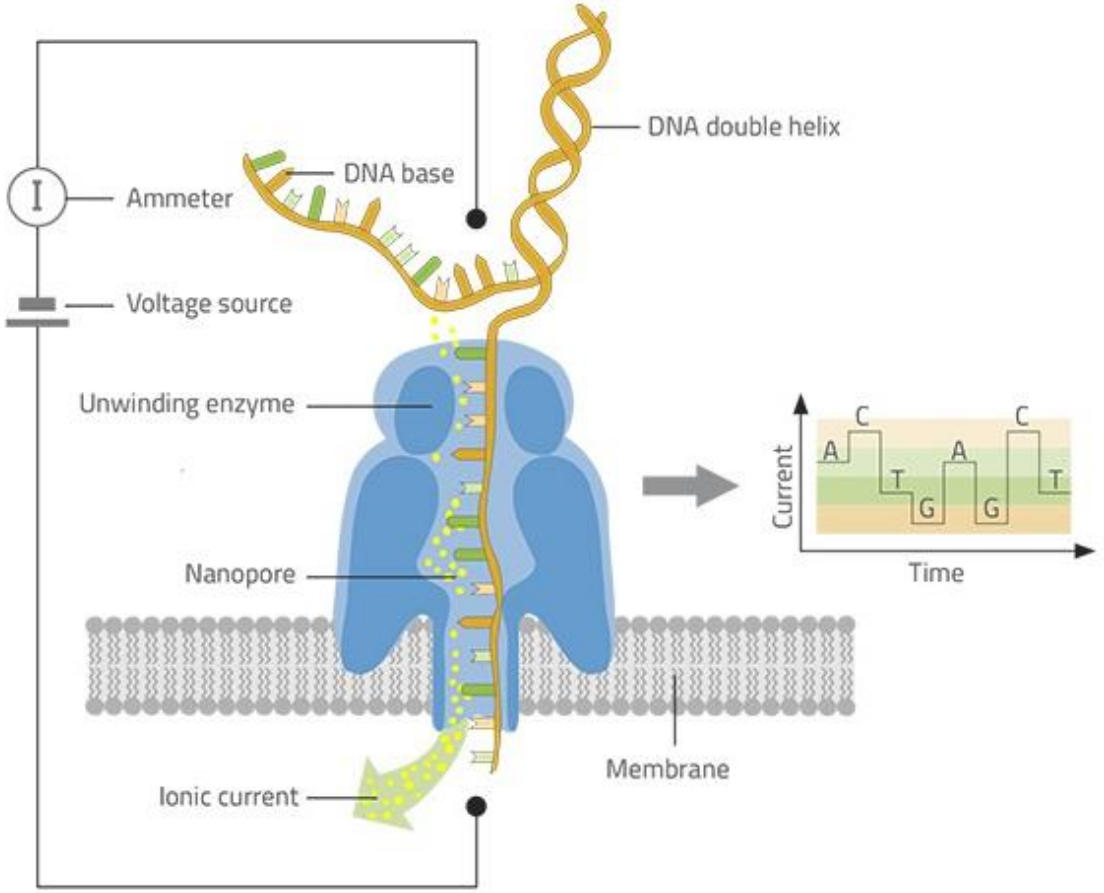
Sequencing platform

MinION

R9 Flow cell->512 channels (810€)

R9.4.1 Flow cell->126 channels (80€)



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Objective:

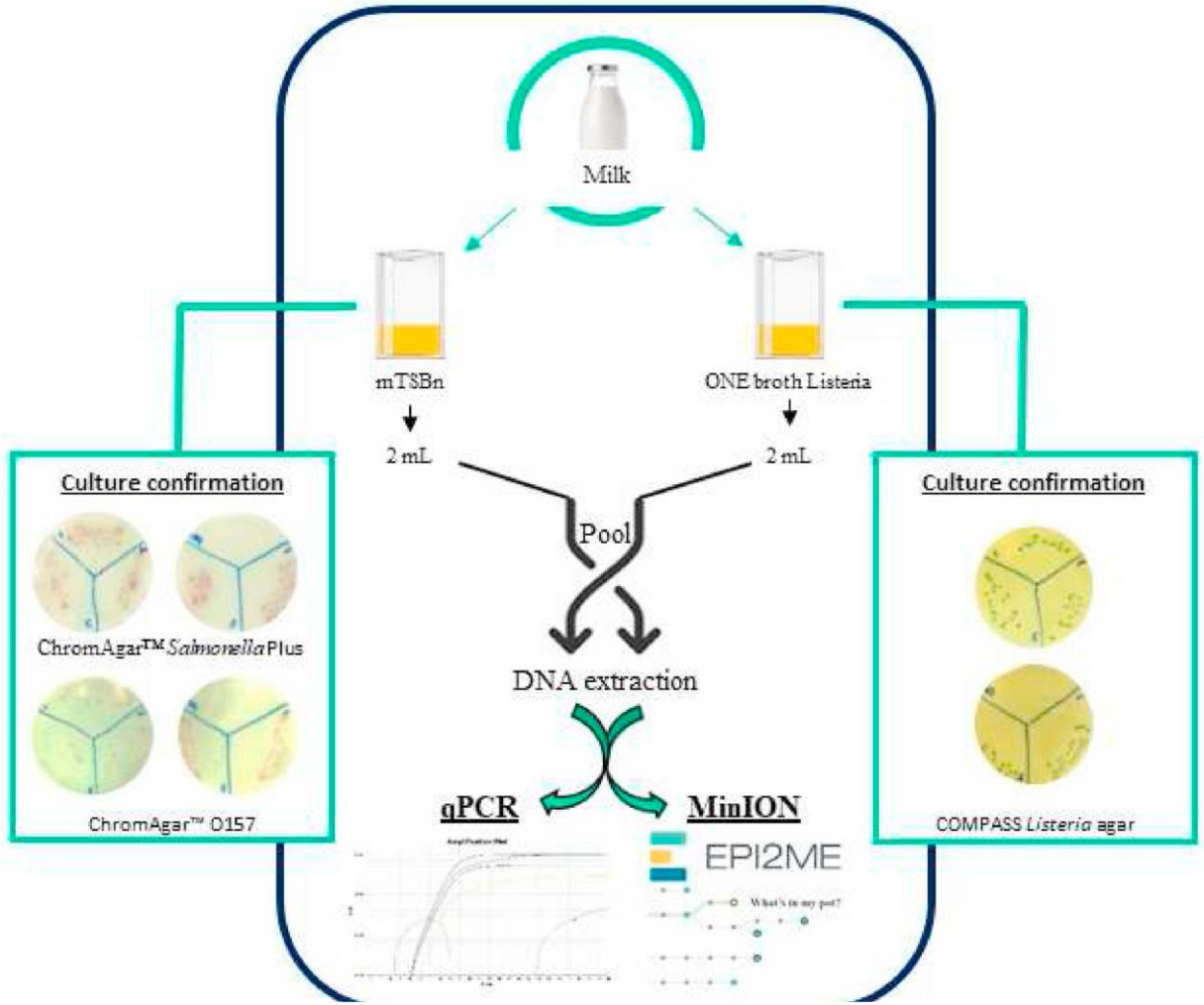
- **Development** of a simple **NGS** methodology
  - Culture
  - Extraction
  - Data analysis

- **Evaluation** in spiked samples
  - Relative sensitivity (SE)
  - Relative specificity (SP)
  - Relative accuracy (AC)
  - Positive Predictive Value (PPV)
  - Negative Predictive Value (NPV)
  - Cohen's kappa (*k*)

Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

Workflow:

- *Salmonella* Typhimurium
- *Salmonella* Enteritidis
- *Escherichia coli* O157:H7



- *Listeria monocytogenes*

Azinheiro et al., 2021. Analytica Chimica Acta, https://doi.org/10.1016/j.aca.2021.339051

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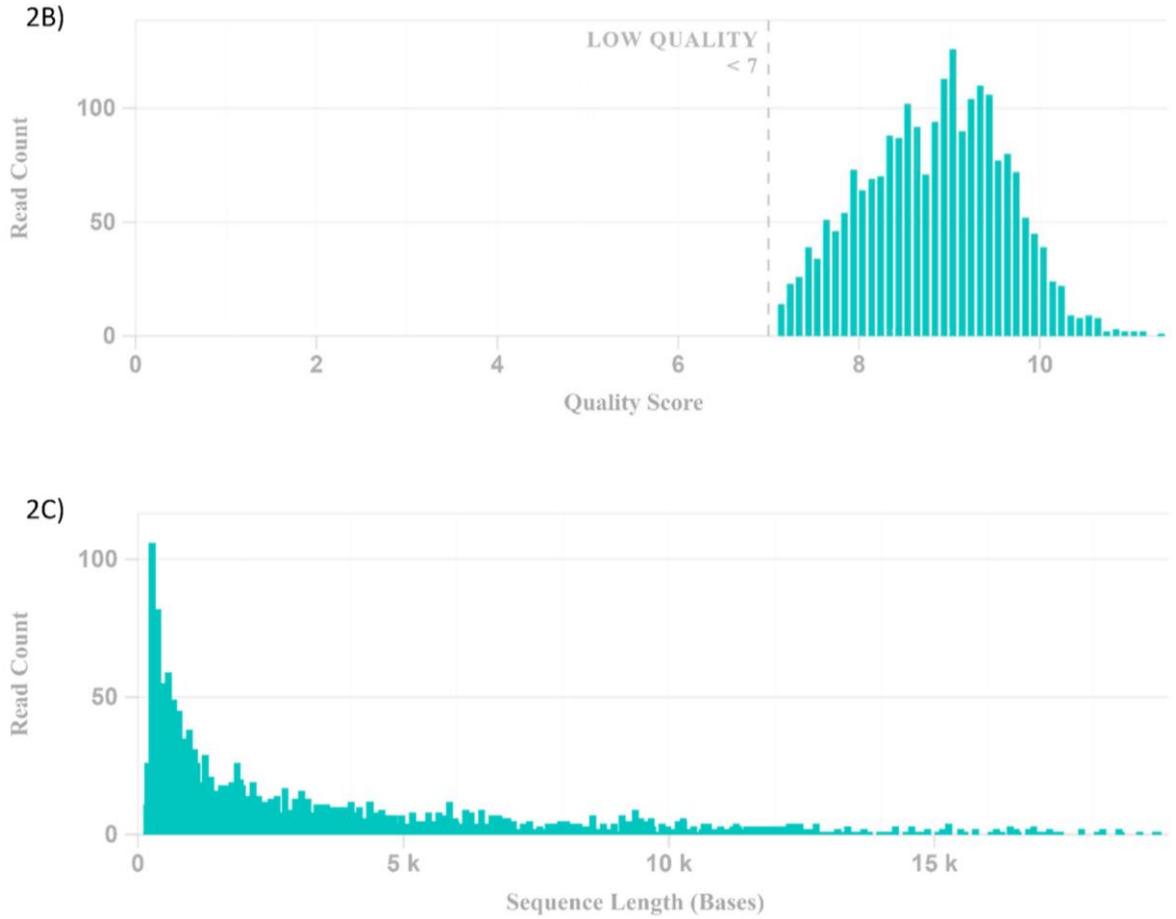
Results:



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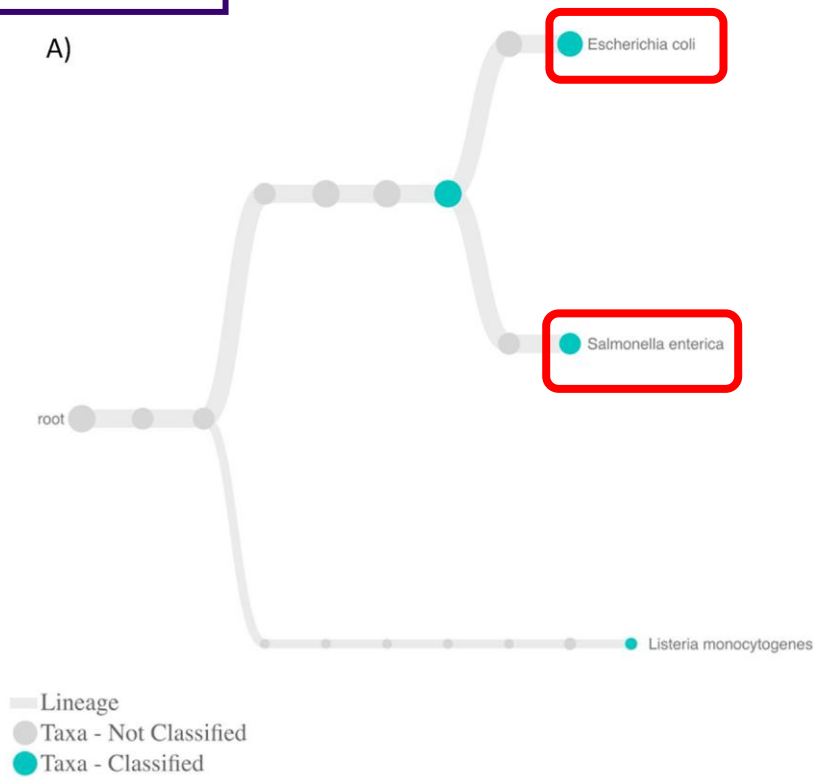
Results:

A)



SPECIES Salmonella enterica

RESULTS | TAXONOMY TREE

READS
2,584

NCBI TAXONOMY ID
28901

Source: NCBI Genomes

LINEAGE
SUPERKINGDOM Bacteria
PHYLUM Proteobacteria
CLASS Gammaproteobacteria
ORDER Enterobacterales
FAMILY Enterobacteriaceae
GENUS Salmonella
SPECIES Salmonella enterica

Of the 2584 reads, 1967 were classified as **Salmonella enterica** and the rest to one of the **98 child taxa:**

Taxon	Reads	Rank
Salmonella enterica subsp. enterica	132	subspecies
Salmonella enterica subsp. enterica serovar Typhimurium	96	no rank
Salmonella enterica subsp. diarizonae	40	subspecies
Salmonella enterica subsp. arizonae	21	subspecies
Salmonella enterica subsp. salamae serovar 55:k:z39 str. 1315K	21	strain
Salmonella enterica subsp. enterica serovar Newport	17	no rank
Salmonella enterica subsp. enterica serovar Enteritidis	15	no rank
Salmonella enterica subsp. enterica serovar Macclesfield str. S-1643	12	strain
Salmonella enterica subsp. enterica serovar Manchester str. ST278	10	strain
Salmonella enterica subsp. enterica serovar Paratyphi A	10	no rank

Azinheiro et al., 2021. Analytica Chimica Acta, https://doi.org/10.1016/j.aca.2021.339051

Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

Results:

Pathogen	Inoculation (CFU/ 25 mL)	N	PA	NA	PD	TP	ND	FP	SE	SP	AC	PPV	NPV	κ
Salmonella spp.	10-100	31	16	15	0	0	0	0	100	100	100	100	100	1.00
S. Typhimurium			11	20	0	0	0	0	100	100	100	100	100	1.00
S. Enteritidis			5	26	0	0	0	0	100	100	100	100	100	1.00
E. coli O157:H7			15	16	0	0	0	0	100	100	100	100	100	1.00
L. monocytogenes			21	10	0	0	1	0	100	100	100	100	100	1.00

N: total number of samples. PA: Positive Agreement. NA: Negative Agreement. PD: Positive Deviation. TP: True Positive. ND: Negative Deviation. FP: False Positive. SE: Relative Sensitivity. SP: Relative Specificity. AC: Relative Accuracy. PPV: Positive Predictive Value. NPV: Negative Predictive Value. k: Cohen's k, interpretation: 0.81-1.00 almost complete concordance according to Altman, D. G. (1990). Practical statistics for medical research. CRC press.

Application of nanopore-based sequencing for multiplex detection of foodborne pathogens

Results:

- LOD
- SE, SP, AC, PPV, NPV 100 %
- *k* 1.00 “almost complete concordance”
- Suitable for Rapid screening

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