# Genomic characterization of *Listeria monocytogenes*

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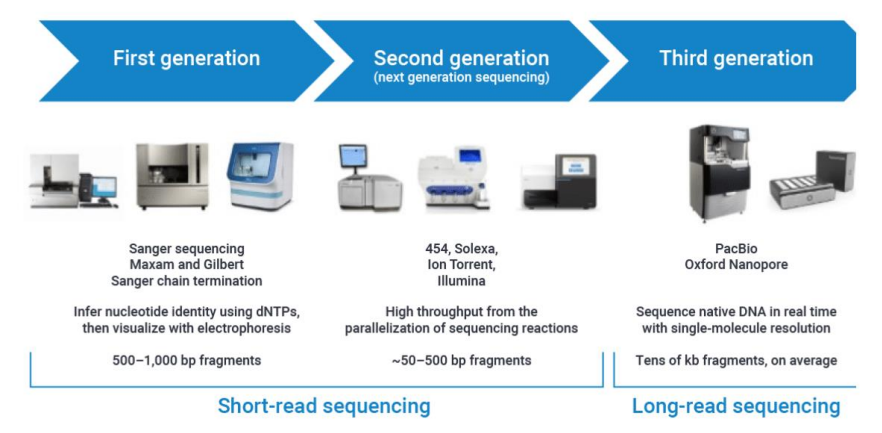
**Istituto Zooprofilattico Sperimentale Abruzzo e Molise "G. Caporale"**

# Introduction

**Genomic characterization:** a laboratory approach used to study about all the genes in a specific cell type and the way those genes interact with each other and with the environment.

**Whole genome sequencing (WGS)** provides the most comprehensive data about a given organism, allowing to obtain its **entire genome**.

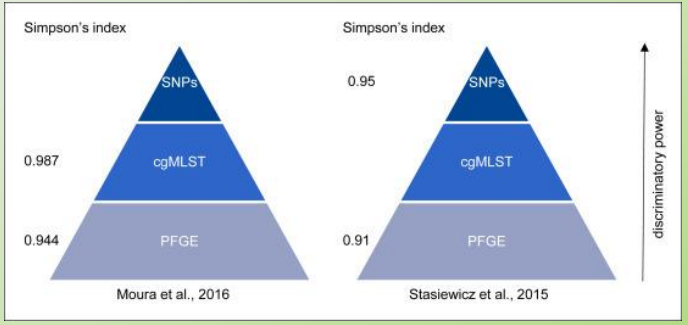
Using **next generation sequencing (NGS)** can deliver large amounts of data in a short amount of time.



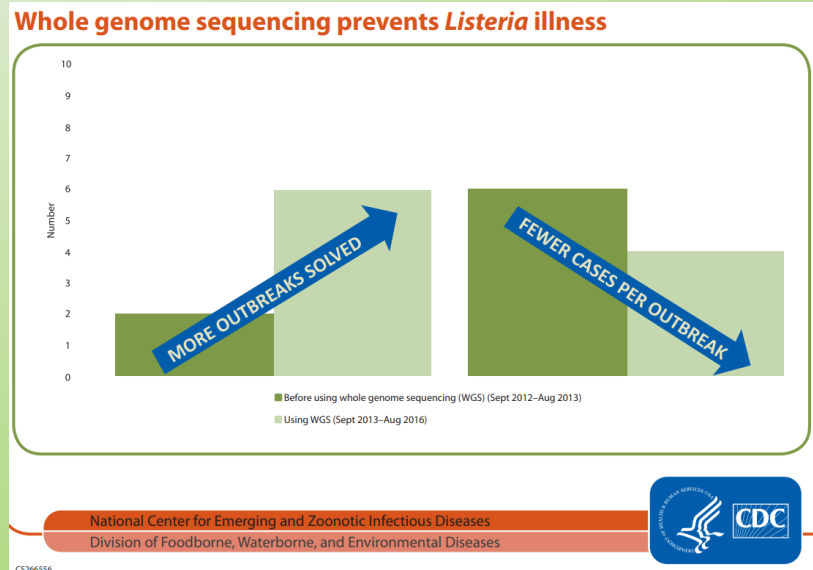
# WGS

Nowadays, WGS is the “gold standard” workflow used in public health and food safety to:

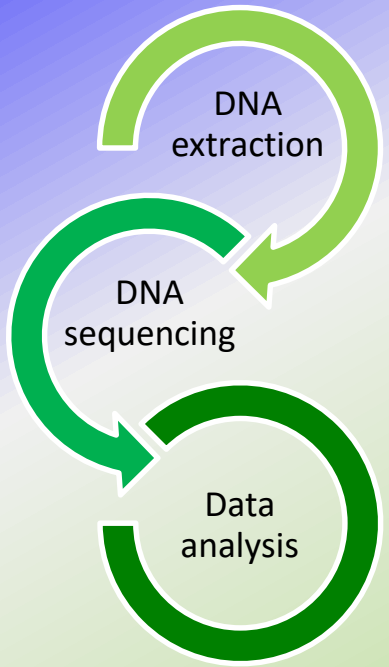
- investigate outbreaks (when they are still contained)
- source attribution for clinical cases
- identify a larger number of clusters (hypothetical outbreaks)

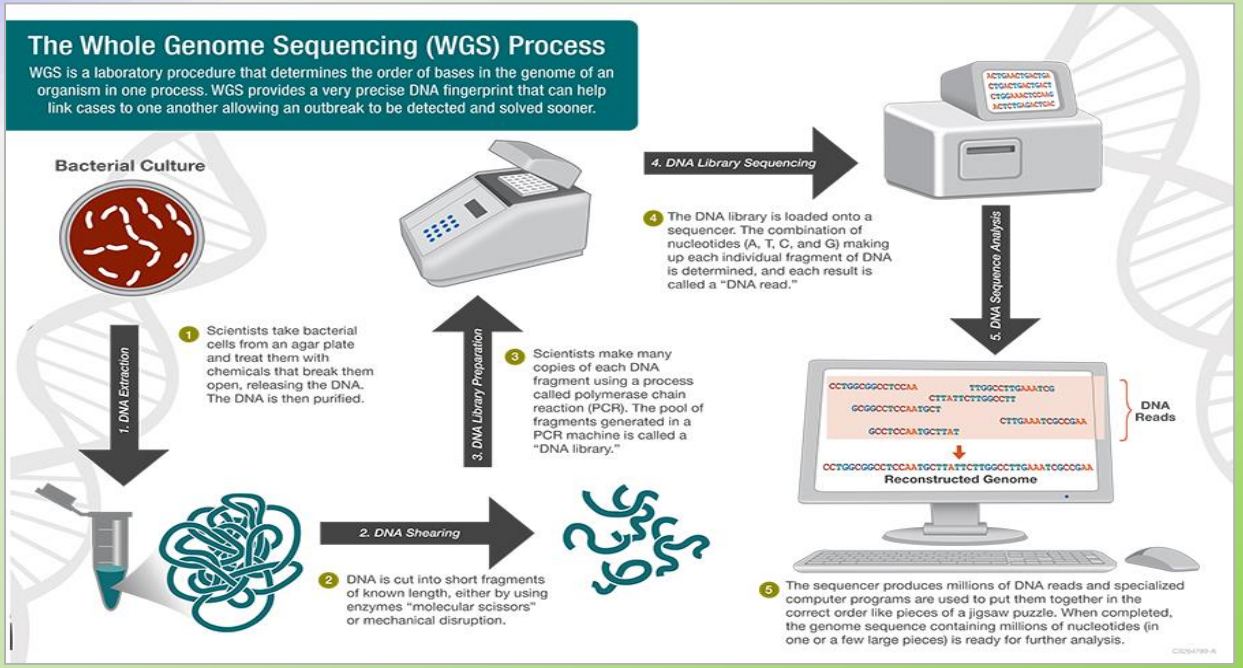




**https://www.cdc.gov/listeria/pdf/whole-genome-sequencing-and-listeria-508c.pdf**



# WGS: WET & DRY



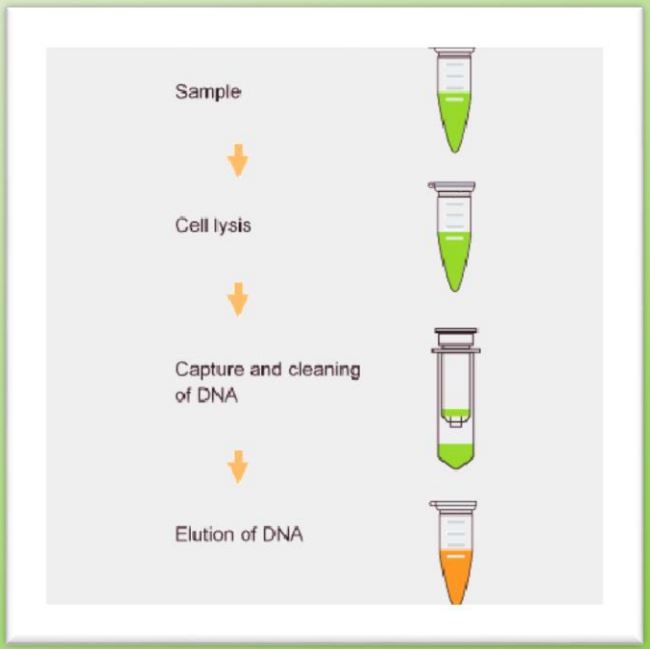

https://www.cdc.gov/pulsenet/pathogens/wgs.html

# DNA EXTRACTION

**Definition**: isolation of DNA by breaking the cell membrane and nuclear membrane with the help of chemicals, enzyme or physical disruptions.

The main steps in DNA extraction includes:

1. preparation of biological material
2. cell lysis and separation of nucleic acids from other cell components
3. DNA precipitation
4. wash and DNA elution



# DNA extraction: our method for NGS

## Columns based DNA extraction

1. In the very first step, the sample is incubated with a **cell lysis buffer** or called a DNA extraction buffer.

2. Along with it, a small amount of **lysozyme** (Gram positive) and **proteinase K** is added to the sample. All the other impurities are removed by centrifugation. DNA remains bounded with silica and other impurities pass through the silica column.

3. Now the DNA can be **washed** twice for improving the purity. The aqueous phase contains the impurities that are discarded by discarding the collection tube.

4. Finally, the DNA is **dissolved** into the AE buffer.

# DNA extraction: our method for NGS

Starting point: **pure culture** from plate or broth

↓

Incubation with **lysozime** 37°C 2h 900rpm

↓

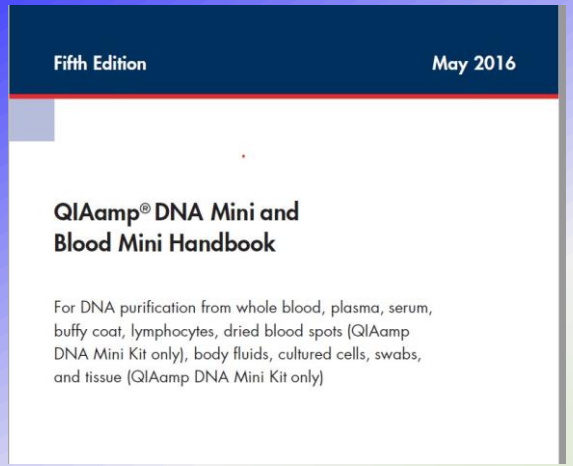
Incubation with **proteinase K** 56°C 1h, vortexing every 20 minutes

↓

**Extraction of DNA** according to the protocol used for bacteria

↓

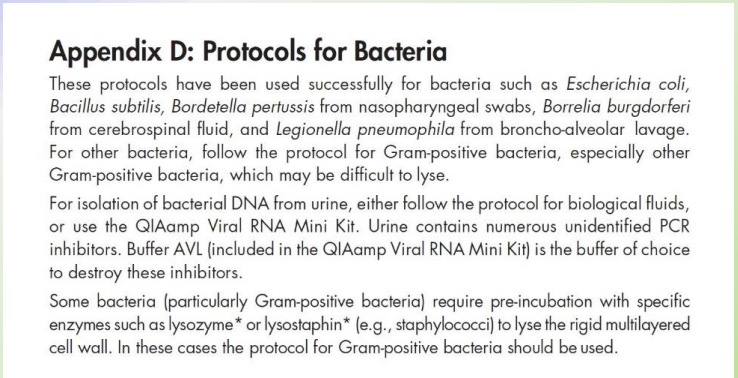
**Dissolving DNA** in AE buffer



Fifth Edition May 2016

**QIAamp® DNA Mini and Blood Mini Handbook**

For DNA purification from whole blood, plasma, serum, buffy coat, lymphocytes, dried blood spots (QIAamp DNA Mini Kit only), body fluids, cultured cells, swabs, and tissue (QIAamp DNA Mini Kit only)

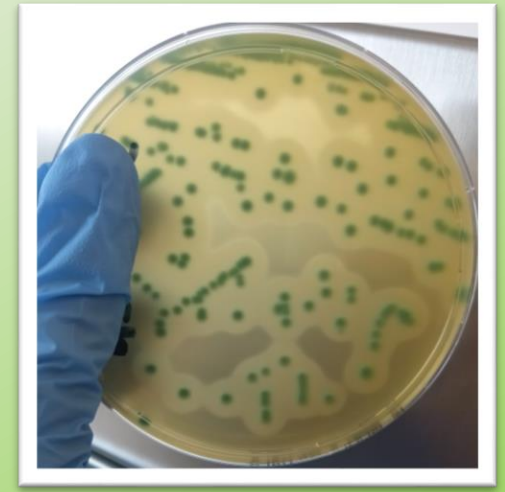


## Appendix D: Protocols for Bacteria

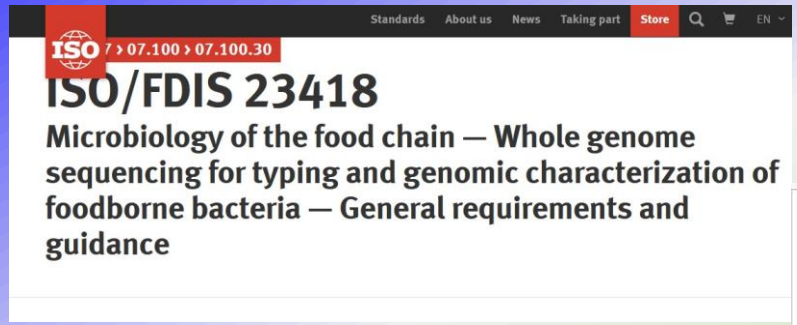
These protocols have been used successfully for bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bordetella pertussis* from nasopharyngeal swabs, *Borrelia burgdorferi* from cerebrospinal fluid, and *Legionella pneumophila* from broncho-alveolar lavage. For other bacteria, follow the protocol for Gram-positive bacteria, especially other Gram-positive bacteria, which may be difficult to lyse.

For isolation of bacterial DNA from urine, either follow the protocol for biological fluids, or use the QIAamp Viral RNA Mini Kit. Urine contains numerous unidentified PCR inhibitors. Buffer AVL (included in the QIAamp Viral RNA Mini Kit) is the buffer of choice to destroy these inhibitors.

Some bacteria (particularly Gram-positive bacteria) require pre-incubation with specific enzymes such as lysozyme* or lysostaphin* (e.g., staphylococci) to lyse the rigid multilayered cell wall. In these cases the protocol for Gram-positive bacteria should be used.



# ISO requirements for DNA




ISO/CD 23418 (E)

**Table A.1 Guidance for development of quality metrics for short and long-read sequencing technologies**

| | | Guidance | |
|---|---|---|---|
| **Process** | **Concern** | **Short read technology** | **Long read technology** |
| **DNA extraction** | cross-contamination; sample integrity | Broth cultures should be started from a single colony of the isolate being tested. | |
| | | DNA integrity is critical, particularly for long read technologies. Care should be taken to avoid fragmentation of genomic DNA during preparation and storage (e.g. through freeze/thawing) | |
| **DNA quality** | Presence of impurities that may negatively impact library construction | Optical density (OD260/280) ratio should be 1.75 - 2.05 and (OD260/230) ratio should be 2.0-2.2 | |
| | low molecular weight DNA may negatively impact library construction | Extraction methods for genomic DNA should be adapted to sequencing platform being used; DNA integrity can be checked on agarose gel or via capillary electrophoresis with appropriate size standards | |
| **DNA quantity** | Insufficient input of genomic DNA may result in substandard sequence library | Input DNA quantity should be carefully determined using a DNA-specific, intercalating dye-based fluorescence quantification method prior to further dilution. Minimum quantity needed will be dependent on library kit/sequencing technology used. If modified, this should be supported by validation. | |
| **DNA fragmentation** | Sub-optimal fragmentation can result in reduced library yield/reduced coverage | Size distribution of sheared DNA samples should be checked using capillary gel electrophoresis based systems. | |

# DNA quality

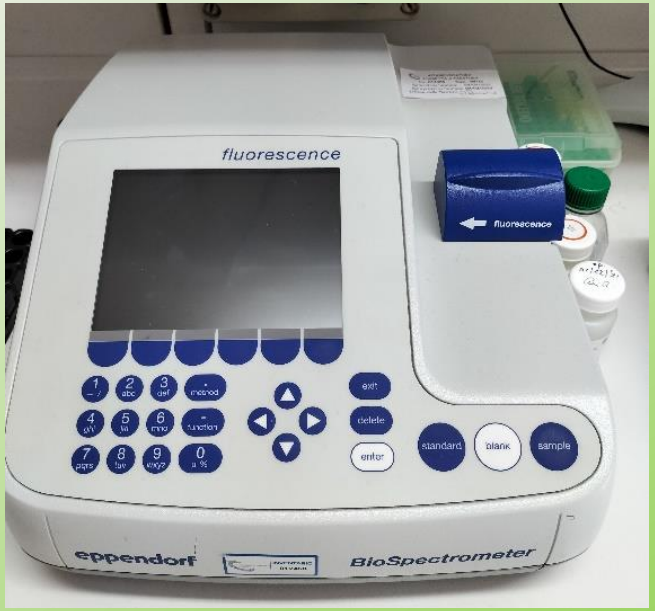
**Quality check:**

- **A260 / A280 ratio**

It determines the degree of protein purification. **A 260/280 ratio of ~1.8 is generally accepted as "pure" for DNA**; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. Inaccurate ratios may also be encountered at very low concentrations (< 10 ng/µl) of nucleic acids.

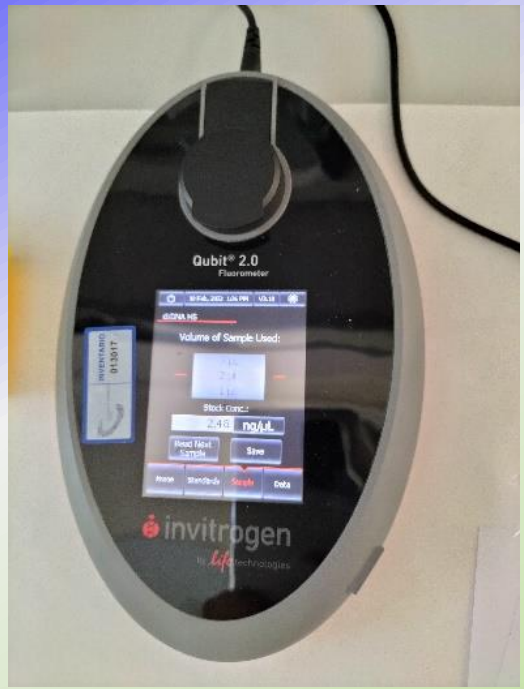
- **A260 / A230 ratio**

Determines the degree of purification of reagent residues. **Expected 260/230 values are commonly in the range of 2.0-2.2**. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorbance at 230 nm.
e.g. EDTA, carbohydrates, guanidine HCL and phenol all have absorbance near 230 nm.



# DNA quantity

**Fluorometric analysis**




The fluorometric measurement of nucleic acids is based upon the use of fluorogenic dyes that bind selectively to DNA or RNA. The signal is measured by fluorometers. Sample is excited with filtered light and the emitted light (at the emission wavelength) is recorded by a detector.

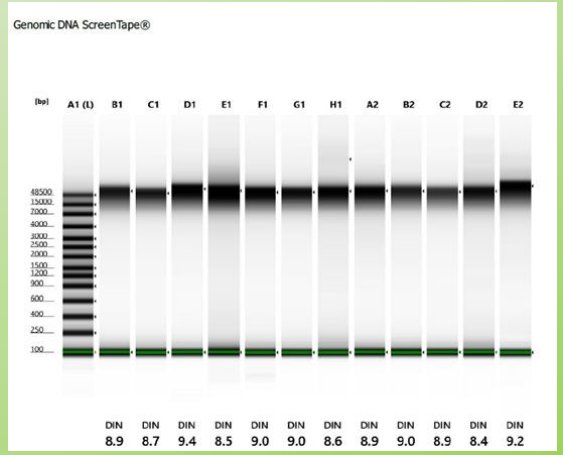
The assay is highly selective for double-stranded DNA (dsDNA) and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The signal is stable for 3 hours.

# DNA integrity

**DNA integrity**

TapeStation Analysis Software assesses the integrity of gDNA samples and assigns the DNA.
Integrity Number (**DIN**), a numerical measure of the gDNA integrity. This value ranges from 1 to 10, where 1 indicates highly degraded gDNA and 10 represents highly intact gDNA.

In our lab **we accept DNA having DIN>7**.



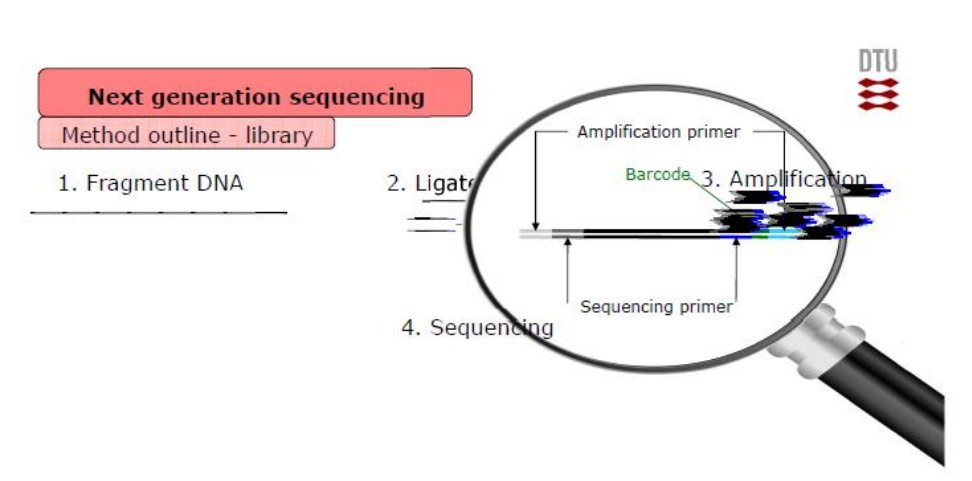
# DNA extraction: work in progress

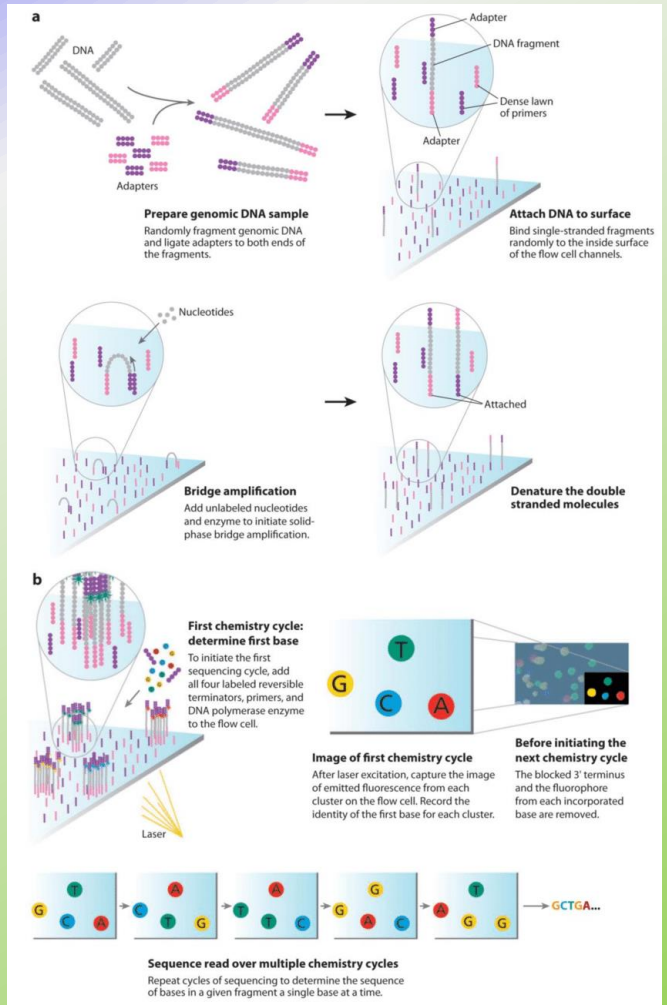
**QIAamp 96 DNA QIAcube HT Kit**

The QIAamp 96 DNA QIAcube HT Kit combines the selective binding properties of a silica-based membrane with a high-throughput 96-well format, and is designed for fully automated, simultaneous processing of 24–96 samples on the QIAcube HT instrument.
Gram + bacteria require lysis steps with lysozyme and proteinase K.
Vacuum based to binds DNA to membrane and later to elute DNA.


- A minimum of 24 samples is required
- A260/A230 is often < 1,7

# DNA sequencing

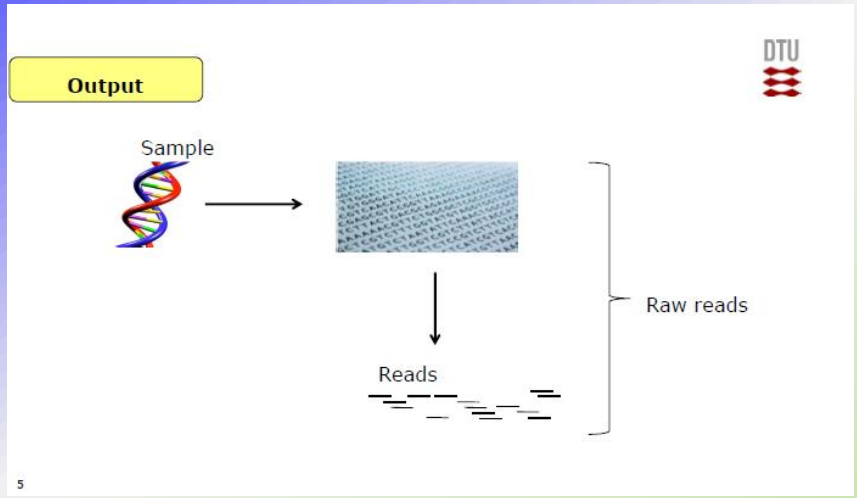



The principle of **Illumina** sequencing process: (A) DNA is converted into an Illumina adapter library and amplified by "bridge amplification" on the surface of the flow cell. (B) Amplified molecules are sequenced by the cycle reversible termination chemistry.

DOI: 10.13140/RG.2.2.29564.08327

# Data analysis





Fastq example:

Quality scores

```
@FCC0CD5ACXX:1:1101:1103:2048#ACGGT/1
ACNGTGTTTTTAGTTATTGTTTTGTTAAGTTGGGTTTTTTGTACCCAATAGCCAACAAGCCGCCTTTATGGCGGTTTTTTTGTGCCTGAAAAGTGGGCGCA
+
_BP`ccceggcegihiiighiifhihfddgfhi^efgfhhhhhegiiiiiiiihiihiggeeccdddcccacWTT^acc[ab_`]`[_b`^BBBBBBBB
@FCC0CD5ACXX:1:1101:1165:2058#ACGTT/1
ACGTTAGCAGAATCGCTTTCTGTTCGTTTTCCACCTGCGACAGACGCACCGGACCACGGTTGGCGAGATCGTCGCGCAGAATATCGGCGGCACGCTGCGAC
+
bb_eeceefeggehhdagfghhiihfghighhffhifhhcghfdhiihafgdceba`a\aaccc^V]^baccaccXaaX^bbcccaac[_X]]a[aacXT
@FCC0CD5ACXX:1:1101:1135:2082#AGCGT/1
AGCGTGACAAACATTTTATTGCGCCCGGTTTTATCCAGCTTGAATGCCTGACGAAAGAAGATGATGGTGACGACGATGGAGAGAACAATCAGCACCAGATT
+
bbbeeeeefggfgiihgiigiiiiiiiffgifgeghiiihhfefffhhhfgh_fhggdgegeaceeacbdcbcc\^aa]``_^bb]bcccccbac_a^bc
@FCC0CD5ACXX:1:1101:1239:2083#AGCGT/1
```

**FASTQ format** is a text-based format to store both a nucleotide sequence and its quality scores.

# Data analysis


Fasta example:

Header/ID

Sequence

```
>gi|218693476|ref|NC_011748.1| Escherichia coli 55989 chromosome, complete genome
GTCTGATAGCAGCTTCTGAACTGGTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAA
ATACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACG
CATTAGCACCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAA
ACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCAT
GCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCGATATTCTG
GAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGG
TGGCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTT
TGCCGAACTTTTGACGGGACTCGCCGCCGCCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTC
GATCAGGAATTTGCCCAAATAAAACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCA
```

**FASTA format** is a text-based format to represent nucleotide sequences (single-letter codes).

FASTA is the main format used to easily manipulate sequences by bioinformatics tools.

# Data analysis

## Table 1 Quality control threshold guidelines for enterica pathogens collected for GenomeTrakr

From: Optimizing open data to support one health: best practices to ensure interoperability of genomic data from bacterial pathogens

| Quality metric | *Salmonella enterica* | *Listeria monocytogenes* | *Escherichia coli* | *Shigella sp.* | *Campylobacter jejuni* | *Vibrio parahaemolyticus* |
|---|---|---|---|---|---|---|
| Average read quality Q score for R1 and R2 | > = 30 | > = 30 | > = 30 | > = 30 | > = 30 | > = 30 |
| Average coverage | > = 30X | > = 20X | > = 40X | > = 40X | > = 20X | > = 40X |
| De novo assembly: Seq. length (Mbp) | ~ 4.3–5.2 | ~ 2.7–3.2 | ~ 4.5–5.9 | ~ 4.0–5.0 | ~ 1.5–1.9 | ~ 4.8–5.5 |
| De novo assembly: no. contigs | <=300 | <=300 | <=500 | <=650 | <=300 | <=300 |

**Timme R.E. et al. Optimizing open data to support one health: best practices to ensure interoperability of genomic data from bacterial pathogens. One Health Outlook 2, 20 (2020). https://doi.org/10.1186/s42522-020-00026-3**

# Multilocus sequence typing (MLST)

MLST is a technique for characterising bacterial isolates using the sequences of internal fragments of **seven house-keeping genes** (**loci**). For each house-keeping gene, the different sequences present within a bacterial species are assigned as distinct alleles (a variant form of a given gene) and designated a random number that just stating that the sequences are different.

An allele profile (for instance: 2, 3, 2, 5, 2, 3, 2) is defined as a **sequence type (ST).**

As far as few years ago, MLST was performed using amplification and Sanger sequencing.
Nowadays , all these results are obtained *in silico*.

| *Locus Listeria* |
|---|
| *abcZ* |
| *bglA* |
| *cat* |
| *dapE* |
| *dat* |
| *ldh* |
| *lhkA* |

| *Clonal Complex* ↑ | Sequence Type |
|---|---|
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 145 |
| CC2 | 145 |
| CC2 | 589 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 2 |
| CC2 | 1430 |

# Multilocus sequence typing (MLST)

**[alexandra.moura@pasteur.fr] Listeria PasteurMLST submission closed - BIGSdb_20220919144659_2003020_65744**

20 settembre 2022 15:34

Da: no reply
A: listeria
Cc: alexandra moura

```
Submission status
=================
ID: BIGSdb_20220919144659_2003020_65744
Data type: genomes
Date submitted: 2022-09-19
Last updated: 2022-09-20
Status: closed
Submitter: Francesco Pomilio (listeria@izs.it), Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise &quot;G.
Caporale
Curator: Alexandra Moura (alexandra.moura@pasteur.fr), Institut Pasteur
Outcome: accepted - data uploaded

Correspondence
==============
Alexandra Moura (2022-09-20 15:33:02+02):

isolate id: 97399

new MLST type: ST3100

new cgMLST type: CT12370


Alexandra Moura (2022-09-20 15:34:21+02):

Thank you for your submission.
```

Frontiers in Microbiology

ORIGINAL RESEARCH
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## Atypical Serogroup IVb-v1 of *Listeria monocytogenes* Assigned to New ST2801, Widely Spread and Persistent in the Environment of a Pork-Meat Producing Plant of Central Italy

OPEN ACCESS

*Fabrizia Guidi[1]*, Cinzia Lorenzetti[1], Gabriella Centorotola[2], Marina Torresi[2], Cesare Cammà[3], Alexandra Chiaverini[2], Francesco Pomilio[2] and Giuliana Blasi[1]*

# Core genome MLST (cgMLST)

**Core genome MLST(cgMLST)** is an extension of the MLST concept to more than seven conserved genes of the species.

✓ It consist of a fixed set of conserved genome-wide genes and are usually species specific.

✓ All public and stable cgMLST schemes are curated by species experts.

✓ It's a gene by gene approach and doesn't require reference genome.

Final result, as for MLST, is the creation of a systematic allele numbering system corresponding to all loci present in the specie's schema.

For *Listeria* the cgMLST is defined on **1748 loci**. Cut off ≥95% of loci (minimum of 1660 loci are needed).

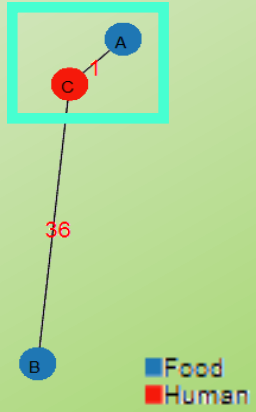
cgMLST schemes may be developed and locally implemented using commercial softwares such as **BioNumerics** (Applied Maths, Sint-Martens-Latem, Belgium), SeqSphere+ (Ridom, Münster, Germany), **BIGSdb** (Pasteur database, France), Center for Genomic Epidemiology (DTU, Denmark) or **in house platform** like GenPat.

# cgMLST clustering: MST

**Minimum spanning tree (MST)** is a graph that connects all the strains together, with the **minimum** possible total edge weight. The minimum spanning tree algorithm is known to be capable of detecting **clusters (≤ 7 allelic differences).**

It allows the analysis of sequence-based typing methods that generate allelic profiles and their associated epidemiological data (**metadata**).

It provides a graphical visualization of core genomic relationships between bacterial isolates.



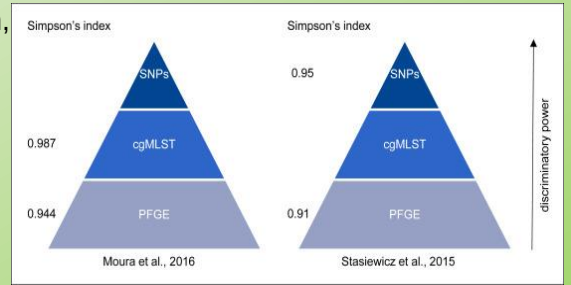
# Single nucleotide polymorphism (SNP)

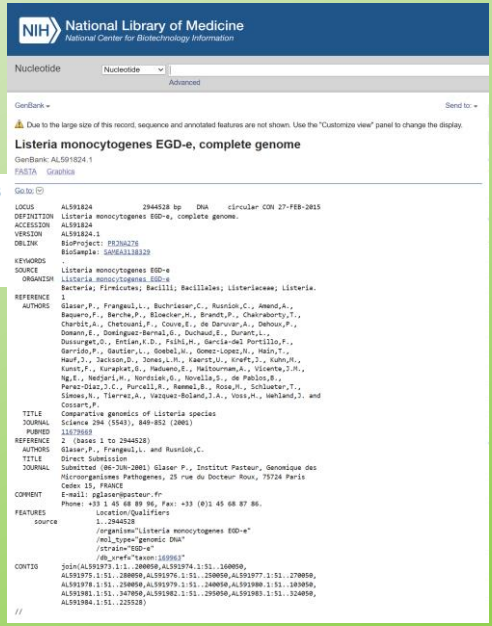
- **Single-nucleotide polymorphism** (**SNP**) is a substitution of a single nucleotide at a specific position in the genome.
- If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then **this variation can be classified as a SNP**.

**Example:**
Isolate 1: ACGTTTACC
Isolate 2: ACCTTTGCC
Isolate 3: ACCTTAGCC

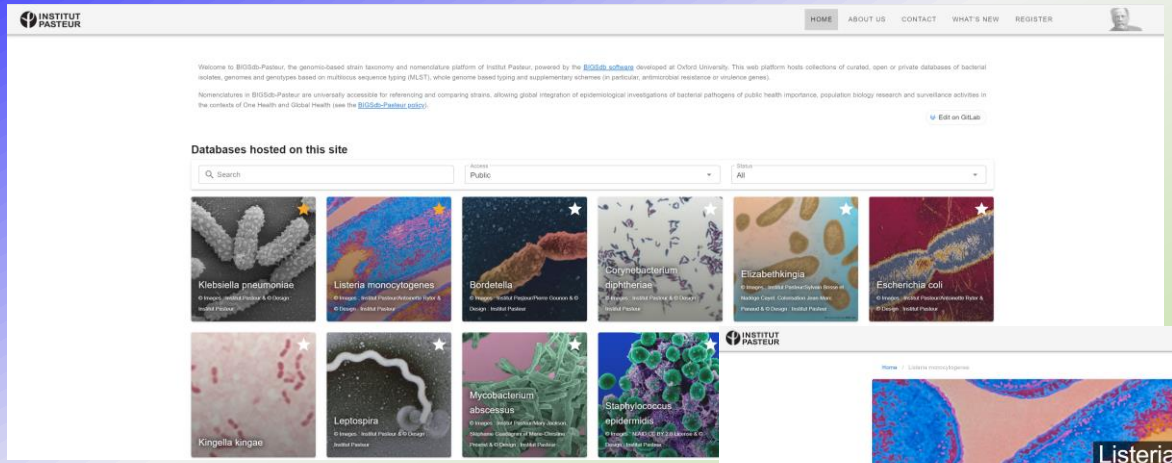
- **SNP genotyping** is the measurement of genetic variations of SNPs between members of a species.
- **It usually requires a reference genome.**
- It can be used to:
  - discriminate the genetic relatedness in a bacterial population,
  - **trace the evolutionary origin of a bacterial species**.

```
LOCUS       AL591824              2944528 bp    DNA     circular CON 27-FEB-2015
DEFINITION  Listeria monocytogenes EGD-e, complete genome.
ACCESSION   AL591824
VERSION     AL591824.1
DBLINK      BioProject: PRJNA276
            BioSample: SAMEA3138329
```

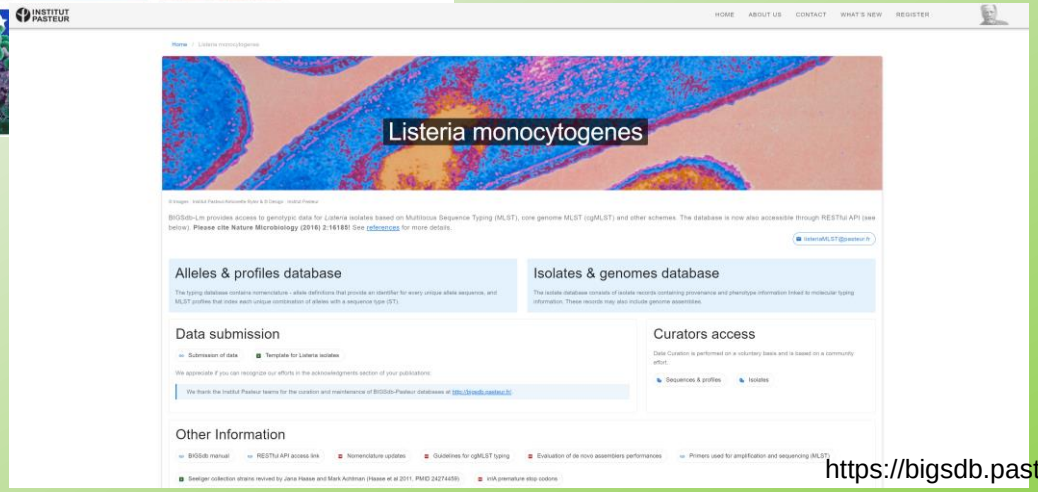


# Database for *in silico* results: Bigsdb Pasteur

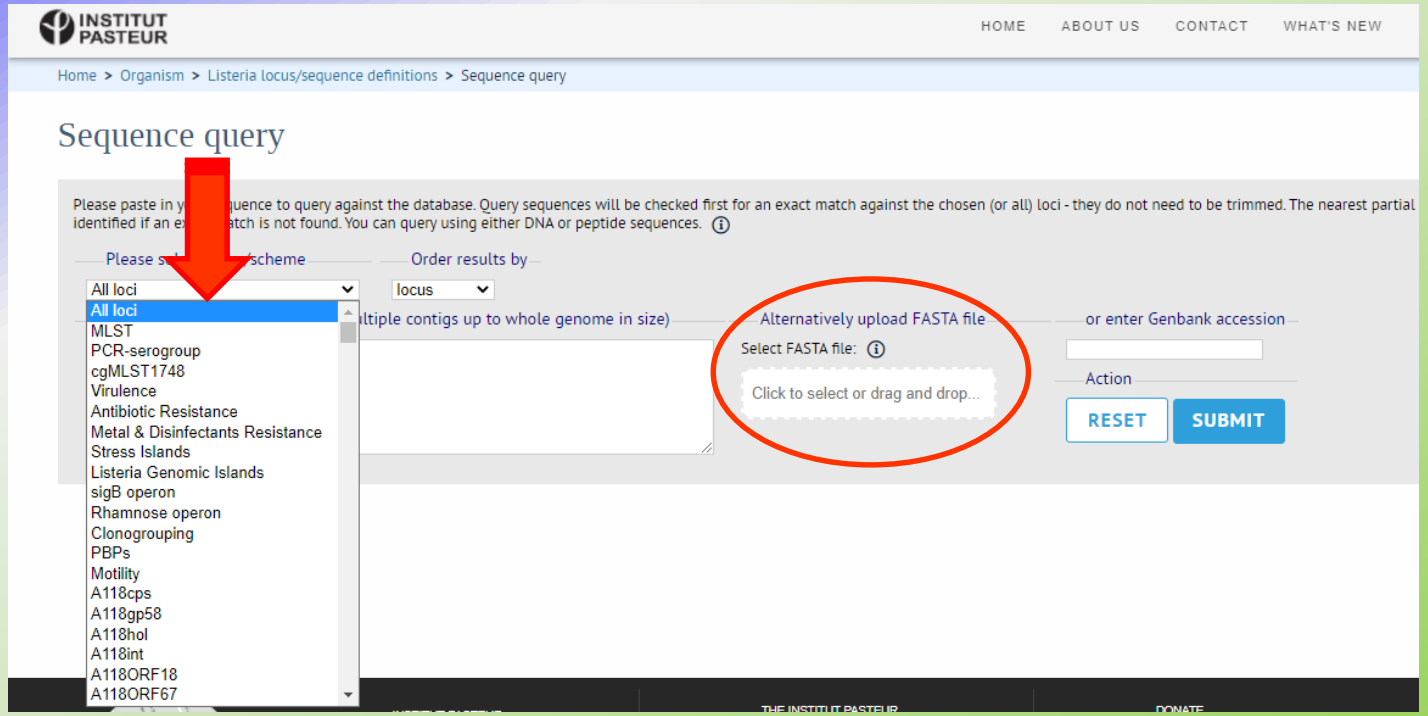



https://bigsdb.pasteur.fr/




https://bigsdb.pasteur.fr/listeria/

# Bigsdb Pasteur:
# *in silico* MLST, cgMLST… and not only



# Bigsdb Pasteur: *in silico* MLST

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7 exact matches found.

| Locus | Allele | Length | Contig | Start position | End position | Flags |
|---|---|---|---|---|---|---|
| abcZ (lmo2752) | 2 | 537 | NODE_2_length_347922_cov_8.428441 | 160005 | 160541 | |
| bglA (lmo0319) | 1 | 399 | NODE_11_length_108064_cov_8.553613 | 107027 | 107425 | |
| cat (lmo2785) | 11 | 486 | NODE_2_length_347922_cov_8.428441 | 203620 | 204105 | |
| dapE (lmo0265) | 3 | 462 | NODE_11_length_108064_cov_8.553613 | 49623 | 50084 | |
| dat (lmo1619) | 3 | 471 | NODE_3_length_342314_cov_6.438290 | 148638 | 149108 | |
| ldh (lmo0210) | 1 | 453 | NODE_8_length_149619_cov_8.590603 | 16766 | 17218 | |
| lhkA (lmo1508) | 7 | 480 | NODE_3_length_342314_cov_6.438290 | 26979 | 27458 | |

Only exact matches are shown above. If a locus does not have an exact match, try querying specifically against that locus to find the closest match.

Text Excel

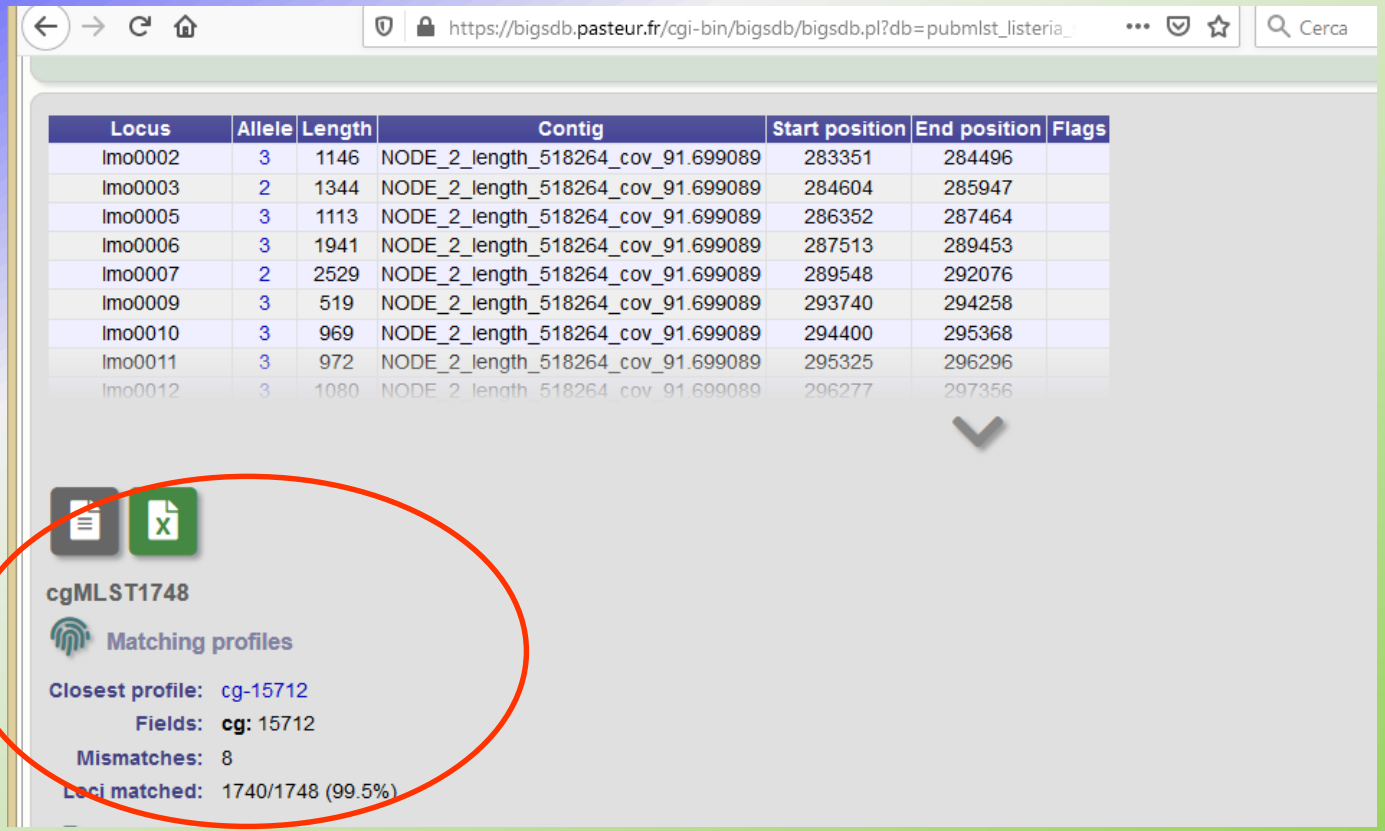
MLST

Matching profile

ST: 5

CC: CC5

# Bigsdb Pasteur:
# *in silico* cgMLST




| Locus | Allele | Length | Contig | Start position | End position | Flags |
|---|---|---|---|---|---|---|
| lmo0002 | 3 | 1146 | NODE_2_length_518264_cov_91.699089 | 283351 | 284496 | |
| lmo0003 | 2 | 1344 | NODE_2_length_518264_cov_91.699089 | 284604 | 285947 | |
| lmo0005 | 3 | 1113 | NODE_2_length_518264_cov_91.699089 | 286352 | 287464 | |
| lmo0006 | 3 | 1941 | NODE_2_length_518264_cov_91.699089 | 287513 | 289453 | |
| lmo0007 | 2 | 2529 | NODE_2_length_518264_cov_91.699089 | 289548 | 292076 | |
| lmo0009 | 3 | 519 | NODE_2_length_518264_cov_91.699089 | 293740 | 294258 | |
| lmo0010 | 3 | 969 | NODE_2_length_518264_cov_91.699089 | 294400 | 295368 | |
| lmo0011 | 3 | 972 | NODE_2_length_518264_cov_91.699089 | 295325 | 296296 | |
| lmo0012 | 3 | 1080 | NODE_2_length_518264_cov_91.699089 | 296277 | 297356 | |

**cgMLST1748**

**Matching profiles**

**Closest profile:** cg-15712
**Fields:** **cg:** 15712
**Mismatches:** 8
**Loci matched:** 1740/1748 (99.5%)

# Bigsdb Pasteur: *in silico* analysis

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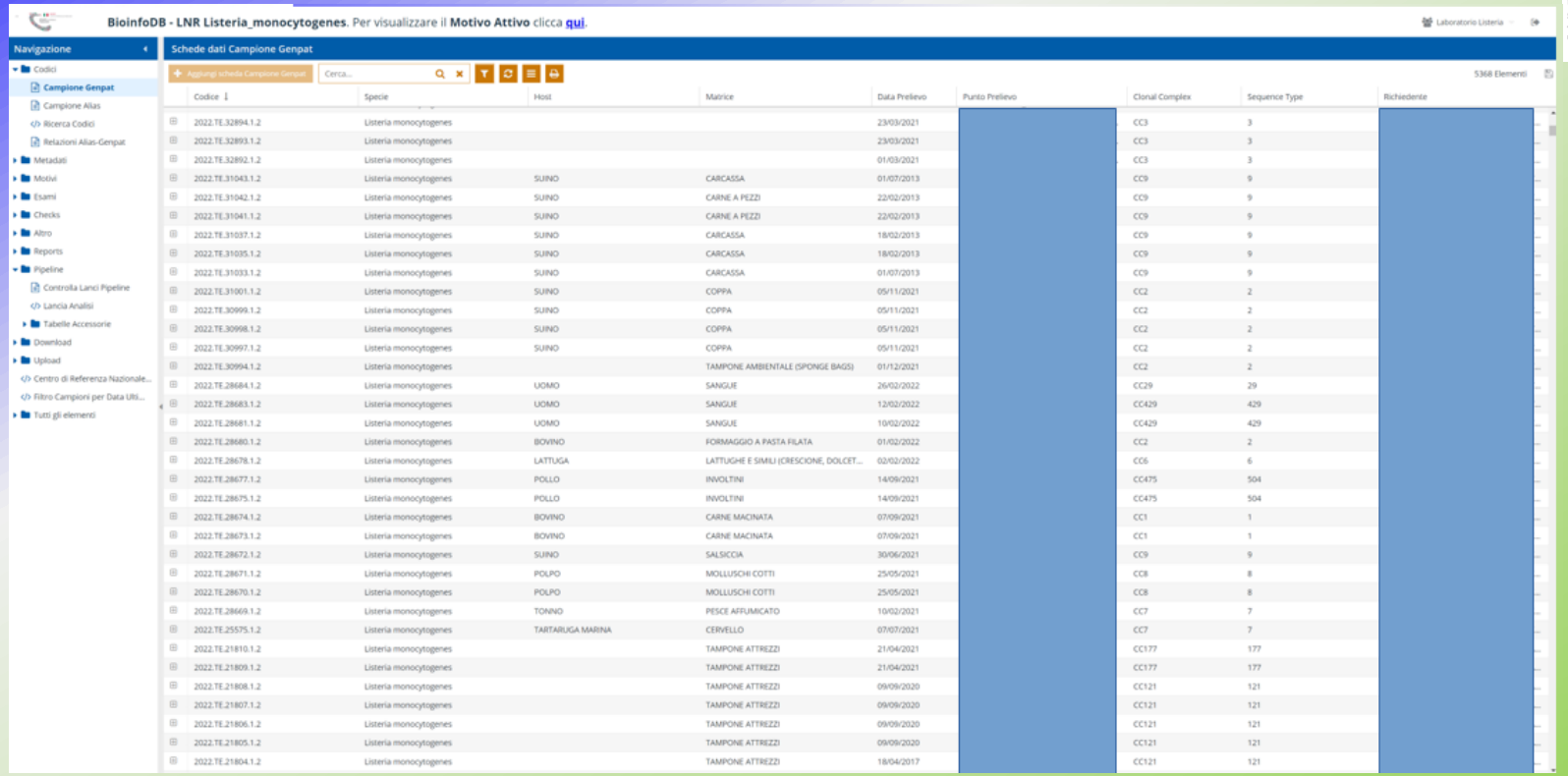
NATIONAL REFERENCE LABORATORY FOR *LISTERIA MONOCYTOGENES*

IZS TERAMO

NATIONAL REFERENCE CENTRE FOR **WHOLE GENOME SEQUENCING OF MICROBIAL PATHOGENS: DATABASE AND BIOINFORMATIC ANALYSIS**

# GENPAT



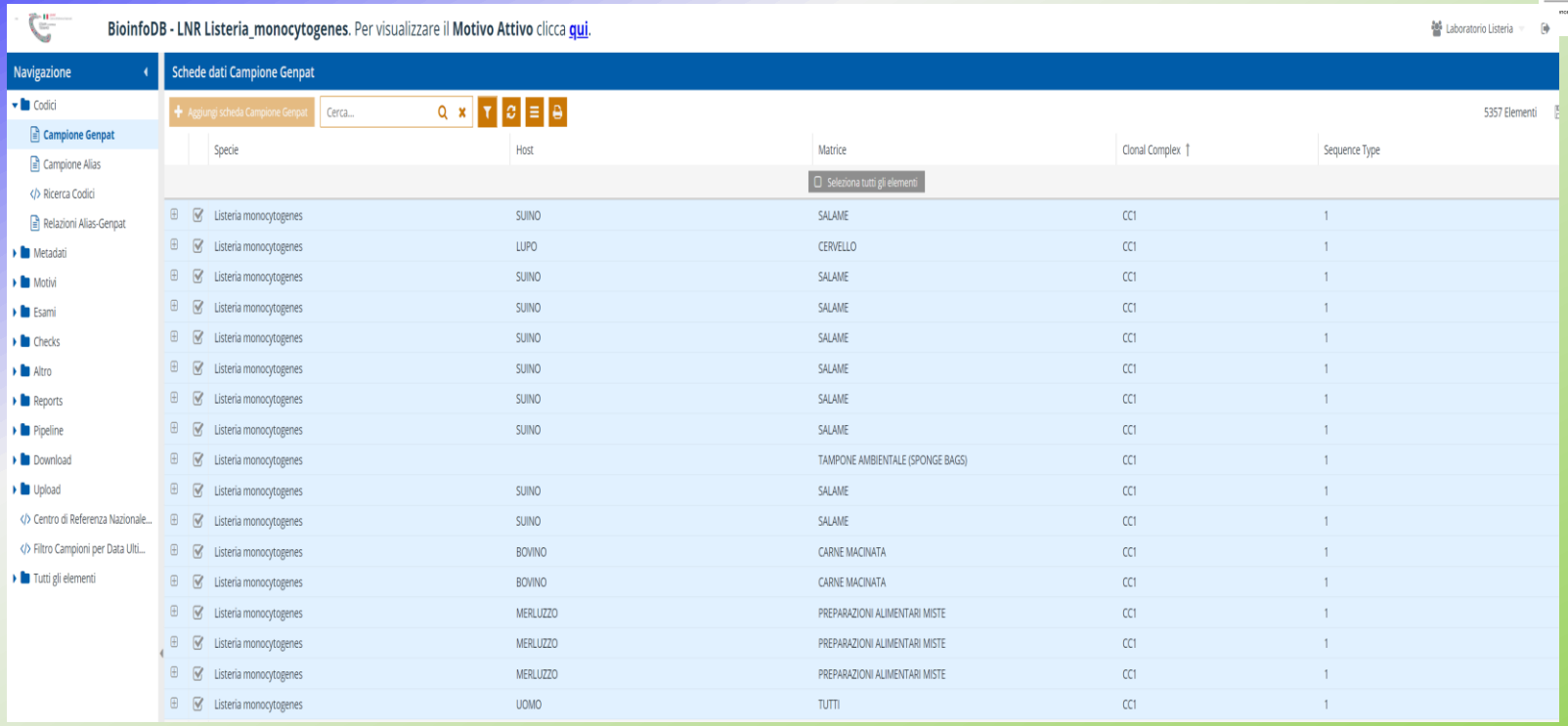

Database of 4000 *Listeria monocytogenes* sequences (from 2015).

**https://genpat.izs.it/.**

WOAH Collaborating Centre for animal production food safety

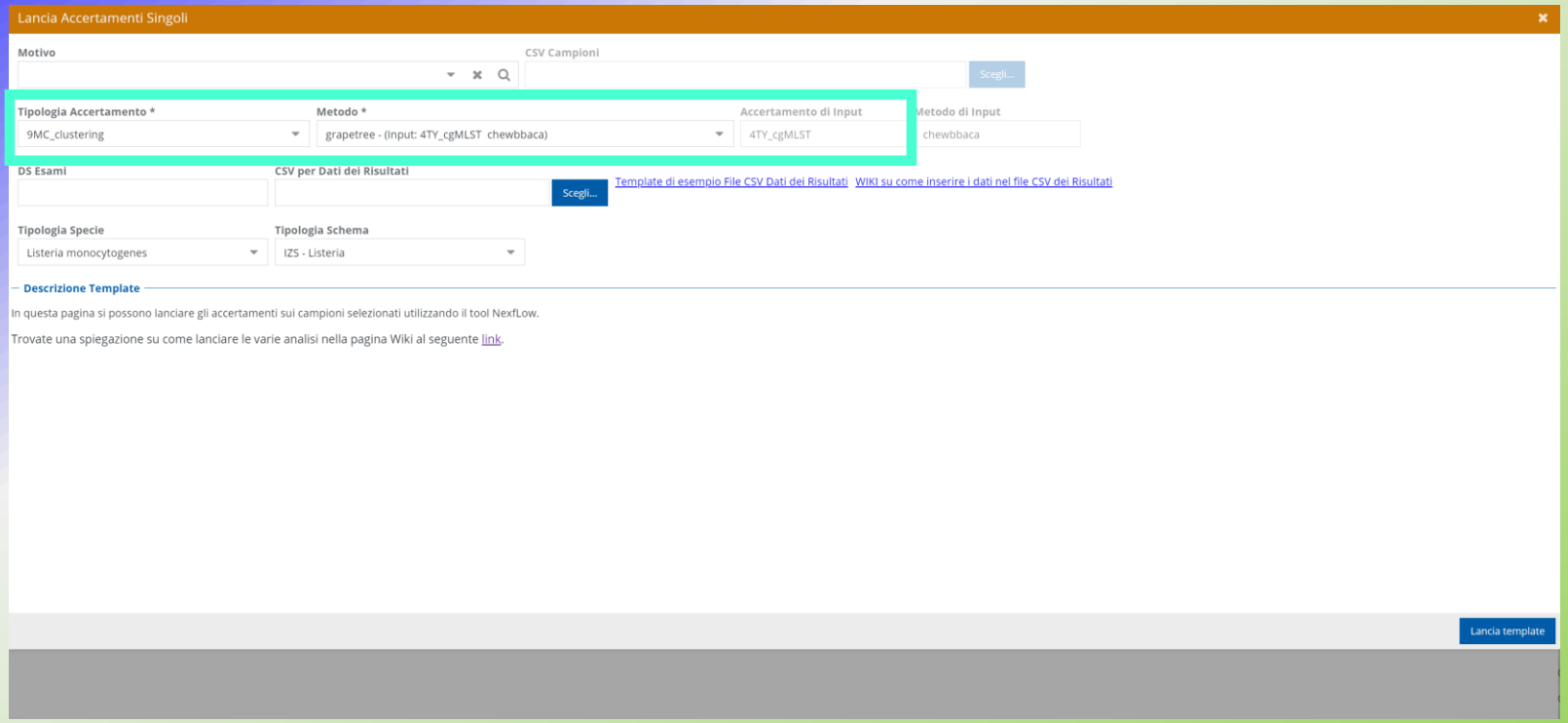
NATIONAL REFERENCE CENTRE GENPAT

# GENPAT




| Specie | Host | Matrice | Clonal Complex | Sequence Type |
|---|---|---|---|---|
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | LUPO | CERVELLO | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | | TAMPONE AMBIENTALE (SPONGE BAGS) | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | SUINO | SALAME | CC1 | 1 |
| Listeria monocytogenes | BOVINO | CARNE MACINATA | CC1 | 1 |
| Listeria monocytogenes | BOVINO | CARNE MACINATA | CC1 | 1 |
| Listeria monocytogenes | MERLUZZO | PREPARAZIONI ALIMENTARI MISTE | CC1 | 1 |
| Listeria monocytogenes | MERLUZZO | PREPARAZIONI ALIMENTARI MISTE | CC1 | 1 |
| Listeria monocytogenes | MERLUZZO | PREPARAZIONI ALIMENTARI MISTE | CC1 | 1 |
| Listeria monocytogenes | UOMO | TUTTI | CC1 | 1 |

# cgMLST Clustering: GenPat IZSAM

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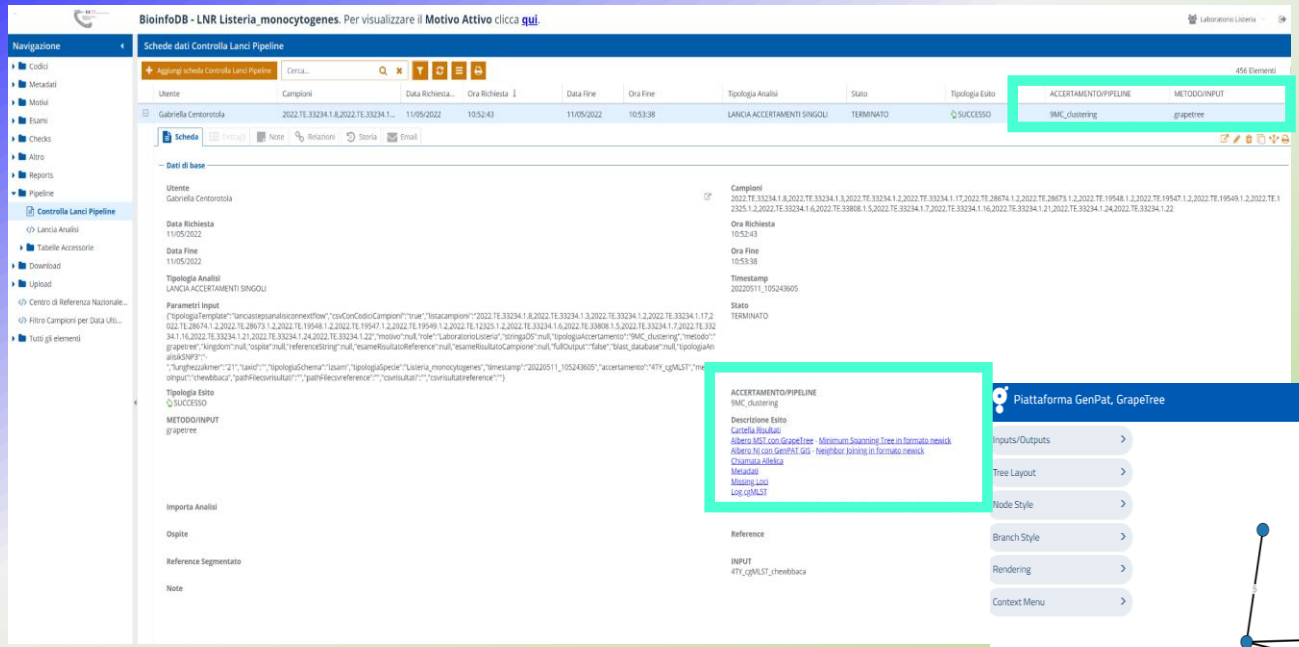
# GENPAT







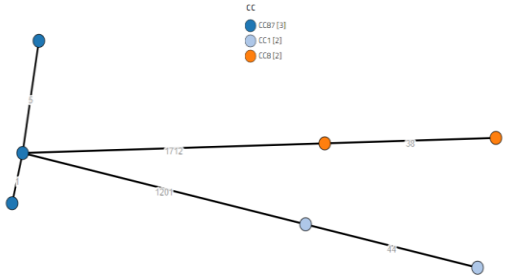
```
chewBBACA version: 2.8.4
Authors: Mickael Silva, Pedro Cerqueira, Rafael Mamede
Github: https://github.com/B-UMMI/chewBBACA
Wiki: https://github.com/B-UMMI/chewBBACA/wiki
Tutorial: https://github.com/B-UMMI/chewBBACA_tutorial
Contacts: imm-bioinfo@medicina.ulisboa.pt

==============================
  chewBBACA - ExtractCgMLST
==============================
Started at: 2022-05-09T11:47:28


Masking missing data...done.
Building presence and absence matrix...done.
Determining genes in the core genome...done.
Determining missing data per genome...done.

Core genome composed of 1740/1748 genes.

Finished at: 2022-05-09T11:47:29
Took  0m 1s.
```



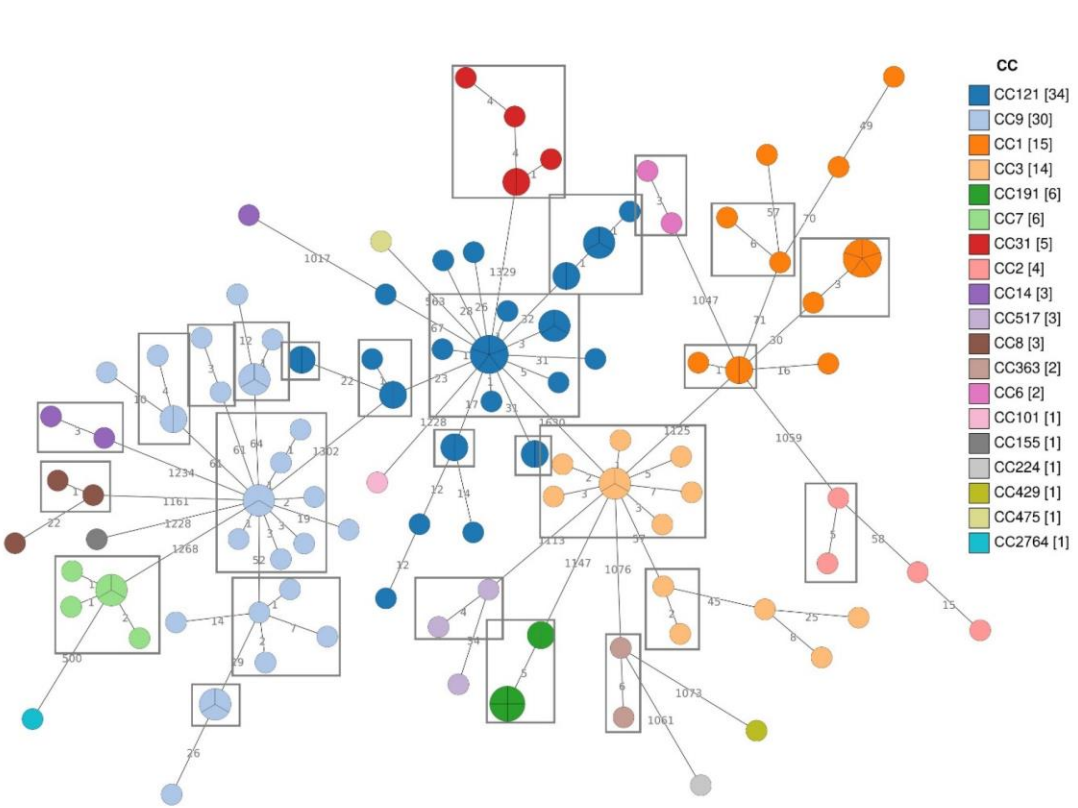



**Figure 1.** Minimum Spanning Tree (MST) based on the cgMLST profiles of 133 *Lm* strains, coloured according to CCs.

**Centorotola G. et al. Intensive Environmental Surveillance Plan for *Listeria monocytogenes* in Food Producing Plants and Retail Stores of Central Italy: Prevalence and Genetic Diversity. *Foods*. 2021;10(8):1944. doi:10.3390/foods10081944**

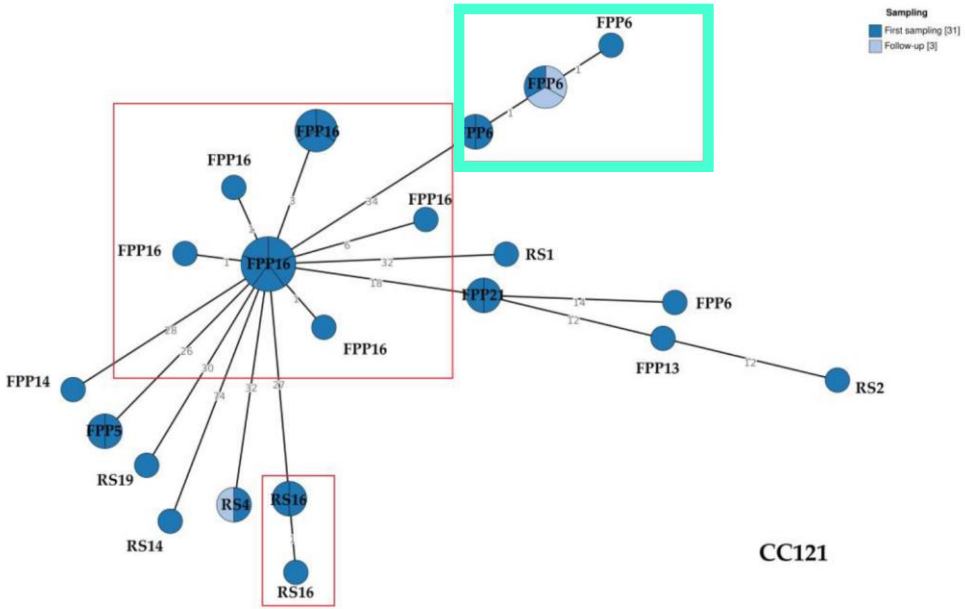
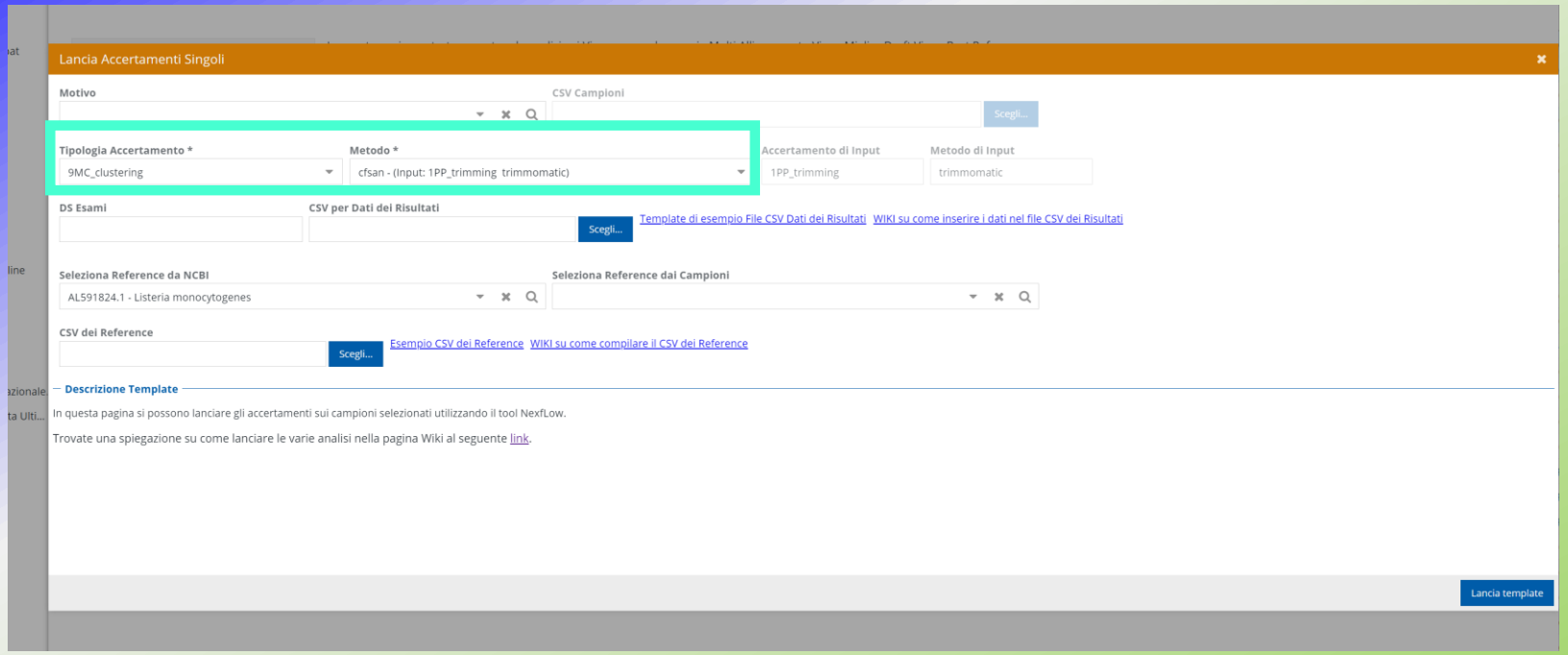



**Figure S1**. Minimum Spanning Tree (MST) based on the cgMLST profiles of CC121 *Lm* strains coloured according to sampling session; the cgMLST clusters containing more than two strains are highlighted in red.

**Centorotola G. et al. Intensive Environmental Surveillance Plan for *Listeria monocytogenes* in Food Producing Plants and Retail Stores of Central Italy: Prevalence and Genetic Diversity. *Foods*. 2021;10(8):1944. doi:10.3390/foods10081944**

# cgMLST Clustering: GenPat IZSAM

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# SNPs clustering: GenPat IZSAM

**Dati di base**

**Utente ***
Gabriella Centorotola

**Data Richiesta**
13/10/2022

**Data Richiesta**
13/10/2022

**Tipologia Analisi**
LANCIA ACCERTAMENTI SINGOLI

**Parametri Input**
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**Tipologia Esito**
Successo

**METODO/INPUT**
cfsan

**Importa Analisi**

**Ospite**

**Reference Segmentato**

**Note**

**Campioni**
2022.TE.5784.1.2,2022.TE.5780.1.2,2022.TE.5775.1.2

**Ora Richiesta**
12:05:04

**Ora Fine**
12:22:49

**Timestamp**
20221013_120504767

**Stato ***
TERMINATO

**Accertamento**
9MC_clustering

**Descrizione Esito**
Cartella Risultati
Albero ML con GenPAT GIS
Maximum Likelihood in formato newick
SNP Matrix
Warnings

**Cartella Analisi (Per visualizzare il link cliccare prima sull'icona della matita in alto a destra.)**
Cartella Analisi

**Reference**
AL591824.1 - Listeria monocytogenes

**Input**
1PP_trimming_trimmomatic

# Conclusion

foods

MDPI

Article

## Intensive Environmental Surveillance Plan for *Listeria monocytogenes* in Food Producing Plants and Retail Stores of Central Italy: Prevalence and Genetic Diversity

Gabriella Centorotola [1], Fabrizia Guidi [2,*], Guglielmo D'Aurizio [3], Romolo Salini [4], Marco Di Domenico [5], Donatella Ottaviani [2], Annalisa Petruzzelli [2], Stefano Fisichella [2], Anna Duranti [2], Franco Tonucci [2], Vicdalia Aniela Acciari [1], Marina Torresi [1], Francesco Pomilio [1] and Giuliana Blasi [2]

pathogens

MDPI

Article

## First Report on the Finding of *Listeria monocytogenes* ST121 Strain in a Dolphin Brain

Yann Sévellec [1], Marina Torresi [2], Benjamin Félix [1], Féderica Palma [1], Gabriella Centorotola [2], Stefano Bilei [3], Matteo Senese [3], Giuliana Terracciano [3], Jean-Charles Leblanc [1], Francesco Pomilio [2] and Sophie Roussel [1,*]

frontiers in Cellular and Infection Microbiology

BRIEF RESEARCH REPORT
published: 20 October 2021
doi: 10.3389/fcimb.2021.765540

## Hyper-Virulent *Listeria monocytogenes* Strains Associated With Respiratory Infections in Central Italy

*Fabrizia Guidi [1*†], Alexandra Chiaverini [2†], Antonella Repetto [3†], Cinzia Lorenzetti [1†], Gabriella Centorotola [2†], Viviana Bazzucchi [1], Barbara Palombo [1†], Antonietta Gattuso [4], Francesco Pomilio [2†] and Giuliana Blasi [1†]*

# Conclusion

microorganisms

MDPI

Article

## Hypo- and Hyper-Virulent *Listeria monocytogenes* Clones Persisting in Two Different Food Processing Plants of Central Italy

Fabrizia Guidi [1,2,*], Massimiliano Orsini [3], Alexandra Chiaverini [4], Marina Torresi [4], Patrizia Centorame [4], Vicdalia Aniela Acciari [4], Romolo Salini [5], Barbara Palombo [1], Giorgio Brandi [2], Giulia Amagliani [2], Giuditta Fiorella Schiavano [6], Francesca Romana Massacci [1], Stefano Fisichella [1], Marco Di Domenico [7], Massimo Ancora [7], Adriano Di Pasquale [7], Anna Duranti [1], Cesare Cammà [7], Francesco Pomilio [4] and Giuliana Blasi [1]

International Journal of Food Microbiology 366 (2022) 109562

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

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## Genetic diversity of *Listeria monocytogenes* strains contaminating food and food producing environment as single based sample in Italy (retrospective study)

Vicdalia Aniela Acciari [a,b,*,1], Anna Ruolo [a,b,1], Marina Torresi [a,b], Lucilla Ricci [a], Antonella Pompei [a,b], Cristina Marfoglia [a,b], Francesca Maria Valente [a,b], Gabriella Centorotola [a,b], Annamaria Conte [a], Romolo Salini [a], Nicola D'Alterio [a,b], Giacomo Migliorati [a,b], Francesco Pomilio [a,b]

frontiers in Microbiology

ORIGINAL RESEARCH
published: 04 November 2021
doi: 10.3389/fmicb.2021.750065

## Phylogenetic Analysis and Genome-Wide Association Study Applied to an Italian *Listeria monocytogenes* Outbreak

*Alexandra Chiaverini[1]*, Fabrizia Guidi[2], Marina Torresi[1], Vicdalia Aniela Acciari[1], Gabriella Centorotola[1], Alessandra Cornacchia[1], Patrizia Centorame[1], Cristina Marfoglia[1], Giuliana Blasi[2], Marco Di Domenico[3], Giacomo Migliorati[1], Sophie Roussel[4], Francesco Pomilio[1] and Yann Sevellec[4]*

# Conclusion


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**Control/Tracking Number:** 2022-A-123-NGS
**Activity:** Abstract
**Current Date/Time:** 7/28/2022 4:50:54 AM

**Whole Genome Sequencing of *Listeria monocytogenes* from Zambian Meat Foods: a Focus on Hyper-virulent Clones**

**Author Block: G. Centorotola**[1], M. W. Ziba[2], A. Cornacchia[1], A. Chiaverini[1], M. Torresi[1], D. D'Angelantonio[1], M. Scacchia[1], P. Fandamu[3], B. Bowa[2], P. Mangambwa[2], G. M. Muuka[3], F. Pomilio[1];
[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, ITALY, [2]Ministry of Fisheries and Livestock, Central Veterinary Research Institute, Lusaka, ZAMBIA, [3]Ministry of Fisheries and Livestock, Department of Veterinary Services, Lusaka, ZAMBIA.

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# THANK YOU!

g.centorotola@izs.it