

Bacterial strain typing:

characterizing a number of strains in detail and ascertaining whether they are derived from a single parental organism, is **a way to identify bacteria at the strain level and to uncover the genetic diversity underlying important phenotypic characteristics**



Uses of Typing methods

1. Epidemiologic usefulness:

1. To investigate the source of different strains in outbreak situations, to check the possibility of laboratory cross-contamination etc.
2. To determine whether the second episode of disease is due to a previously isolated strain or to a newly infecting strain, and
3. To determine whether an infection is caused by more than one strain of the organism

2. As an important infection control tool: to

monitor the prevalence of certain strains within a healthcare institution or to investigate if a cluster of infections are unrelated or part of an outbreak



A: Phenotypic Typing Methods: Phenotyping techniques detect characteristics expressed by the microorganism. They are based on biochemical, antigenic or susceptibility (to phages or antimicrobial agents) properties of the organism.

1. Biotyping: Based on metabolic characteristics expressed by an isolate; referred to as 'biotypes'

2. Serotyping: Based on antigenic determinants expressed by the microorganism; referred to as 'serotypes'. O-antigen, K-antigen H-antigen

3. Phage typing: Based on the pattern of resistance or susceptibility to a standard set of phages; referred to as 'phage types'.

4. Resistotyping: Based on the resistance or susceptibility of the isolates against a set of arbitrarily chosen chemical agents

5. Bacteriocin typing: Based on the susceptibility to a set of bacterial peptides (bacteriocin) produced by certain bacteria.

B: Molecular Typing Methods: Molecular techniques are based on the analysis of chromosomal or extrachromosomal genetic elements (such as plasmid) of the organism.

In recent years a plethora of molecular-typing methods have appeared based on the analysis of fragments of DNA split by specific restriction enzymes. Their discriminatory powers and complexity vary widely. With the advancement of molecular epidemiology, a single machine is now able to generate a wealth of information needed to detect, monitor and control new threats such as drug resistance and the emergence of new pathogens. With the widespread use of molecular typing methods, phenotyping typing methods are now being obsolete.

• **Commonly used molecular typing methods are as follows:**

1. Amplified fragment length polymorphism (AFLP)
2. Enterobacterial repetitive intergenic consensus (ERIC)-PCR
3. Multilocus sequence typing (MLST)
4. Multilocus variable-number tandem repeat analysis (MLVA)
5. Pulsed-field gel electrophoresis (PFGE)
6. PCR Ribotyping (agarose based or sequence-based)
7. Repetitive element PCR typing
8. Restriction endonuclease analysis (REA)
9. Surface layer protein A gene sequence typing (slpAST)
10. Whole-genome sequencing (WGS)



Typing methods:
pattern of bands or
DNA sequences



Bacterial typing methods



TYPING



FINGERPRINTING

The **discriminating power** of a method defines the level of correlation of strains in an epidemiological investigation

Typing methods: bands or sequences

Pattern

REA: Restriction endonuclease analysis
Rep-PCR: Repetitive element PCR typing
ERIC-PCR: Enterobacterial repetitive intergenic consensus
AFLP: Amplified fragment length polymorphism
PFGE: Pulsed-Field Gel Electrophoresis
MLVA :Multiple-Locus Variable-number tandem-repeat Analysis
PCR Ribotyping (agarose based or sequence-based)

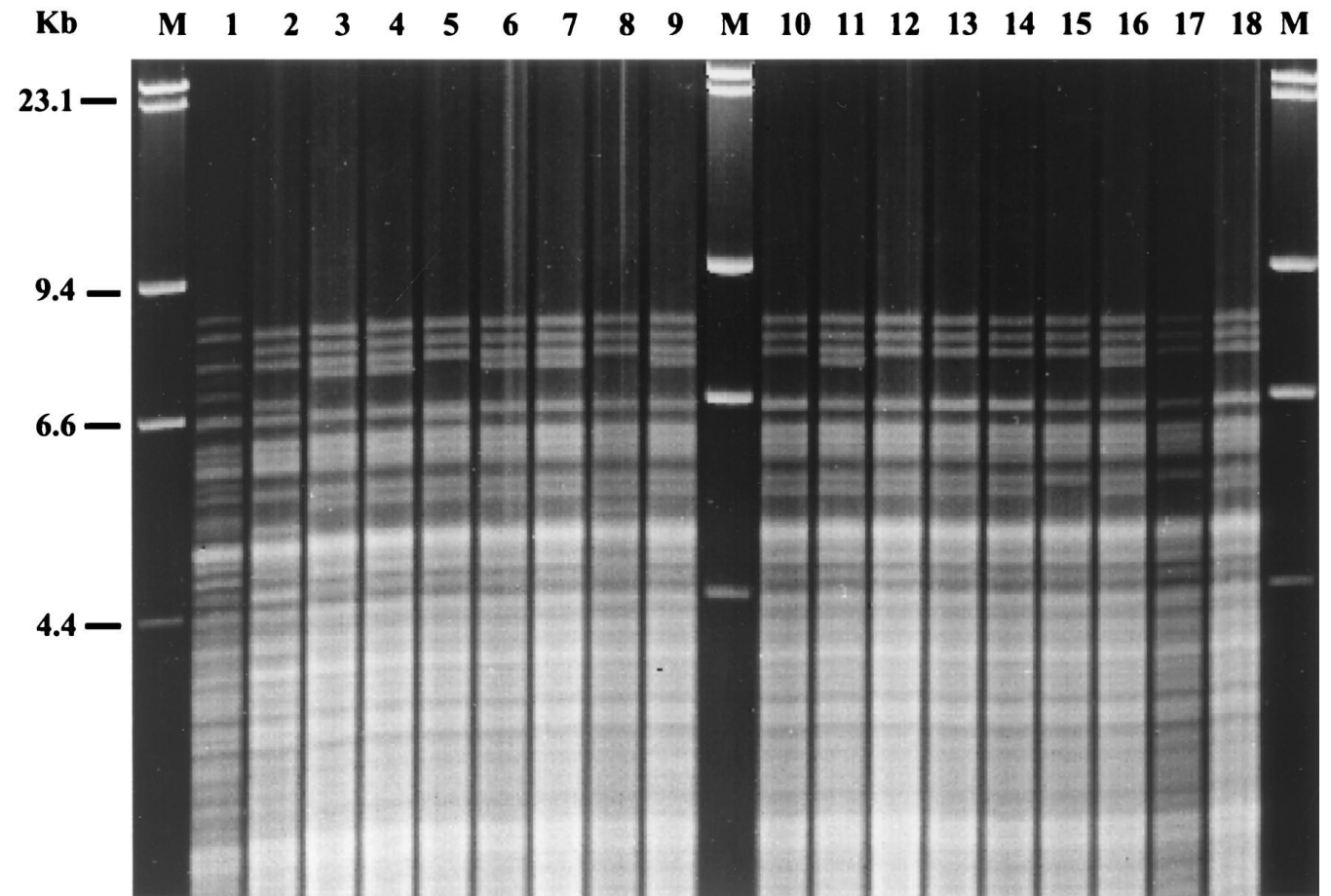
MLST: MultiLocus Sequence Typing
slpAST:Surface layer protein A gene sequence typing
WGS: Whole Genome Sequencing

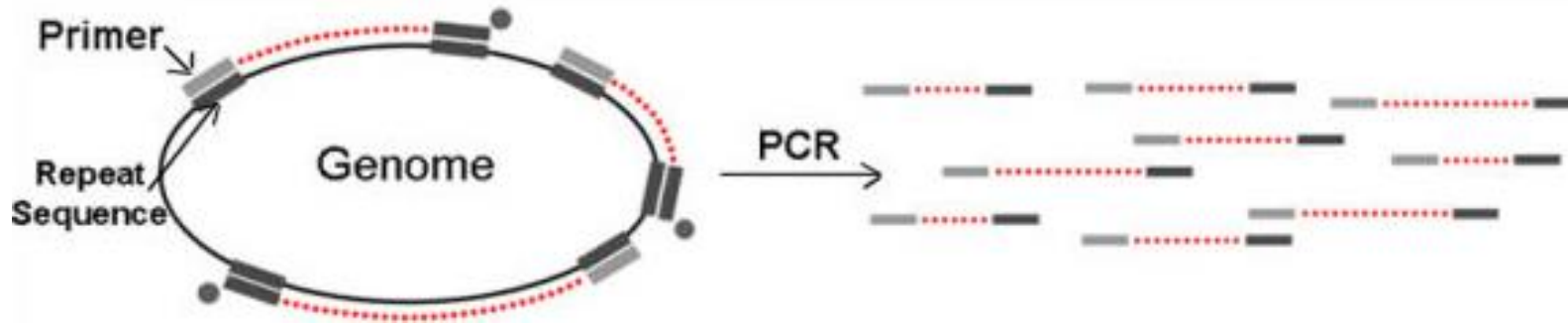
DNA sequences

REA: Restriction endonuclease analysis

Restriction endonuclease analyses (REAs) **constitute the only inexpensive molecular approach capable of typing and characterizing all strains based on their entire genome.** However, the application of this method is limited by the need for time-consuming and labor-intensive procedures.

Digestion of a preparation of genomic DNA and gel electrophoresis.





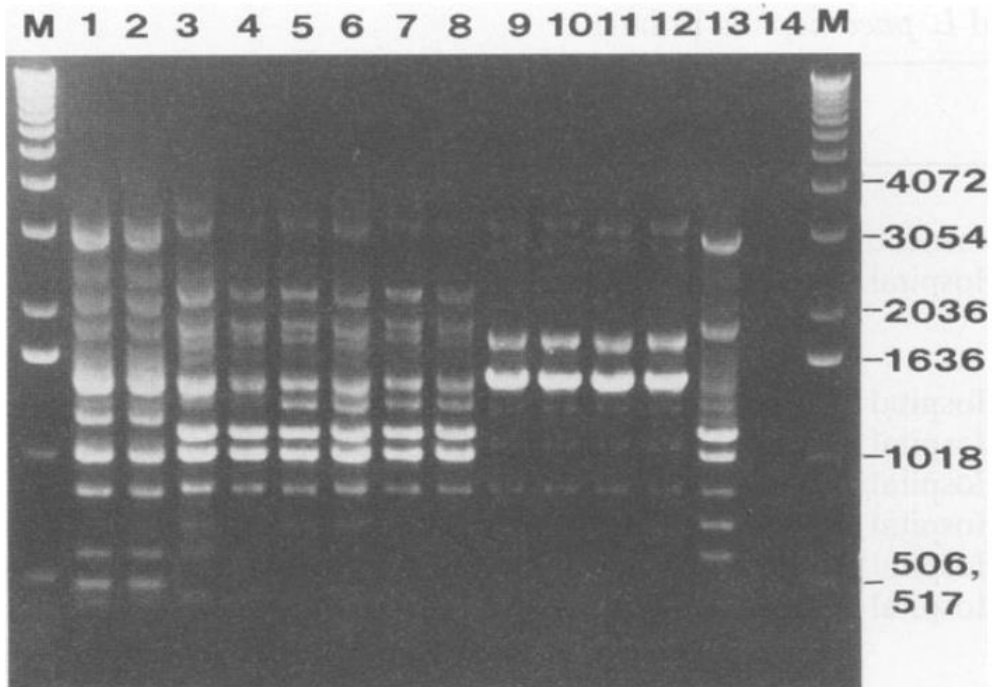
Rep-PCR: Repetitive extragenic palindromic PCR

It is based on the presence of regions of highly conserved and randomly interdispersed repetitive DNA in the genome of a bacterium. The number and location of these regions varies within strains that show differences at the genomic level. The primers are found within the regions and are then generated a series of fragments that will have for each strain length and a defined number

Enterobacterial repetitive intergenic consensus (ERIC) sequences are 127-bp imperfect palindromes that occur in multiple copies in the genomes of enteric bacteria and vibrios

```

          10      20      30      40      50      60
          |      |      |      |      |      |
5' : TATACCCAAAATAATTTCGAGTTGCAGCAAGGCCGCAAGTGAGTGAAT-CCCCAGGAGCTTACAT
      |||||  |||  |||:|:|:|:|  |  |  |:|  |:|  :  |||||  :|||  )
3' : ATATGGGCAGTATAAAGTTCGACGTCGACGCAACCCGACGCAAGCGAGTGGGGTCAGTGAATGAA
          |      |      |      |      |      |
          120    110    100    90     80     70
  
```



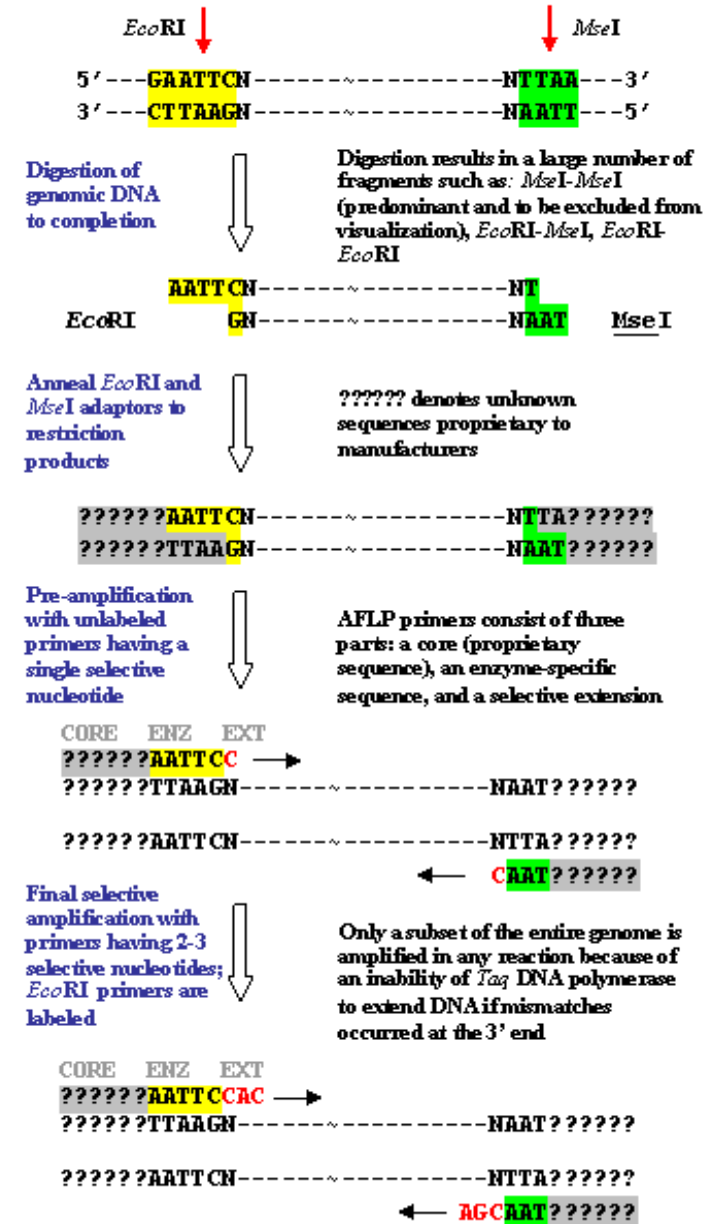
Genomic DNA of different strains were amplified by PCR using specific primers based on repeated rep sequences.

The products obtained by PCR are then separated on agarose gel and allow to identify similar / different strains e.g. (1,2)(3,4)(5-8)(9-12)13.

Amplified fragment length polymorphism (AFLP) is a PCR-based technique that uses selective amplification of a subset of digested DNA fragments to generate and compare unique fingerprints for genomes of interest. The power of this method relies mainly in that it does not require prior information regarding the targeted genome, as well as in its high reproducibility and sensitivity for detecting polymorphism at the level of DNA sequence.

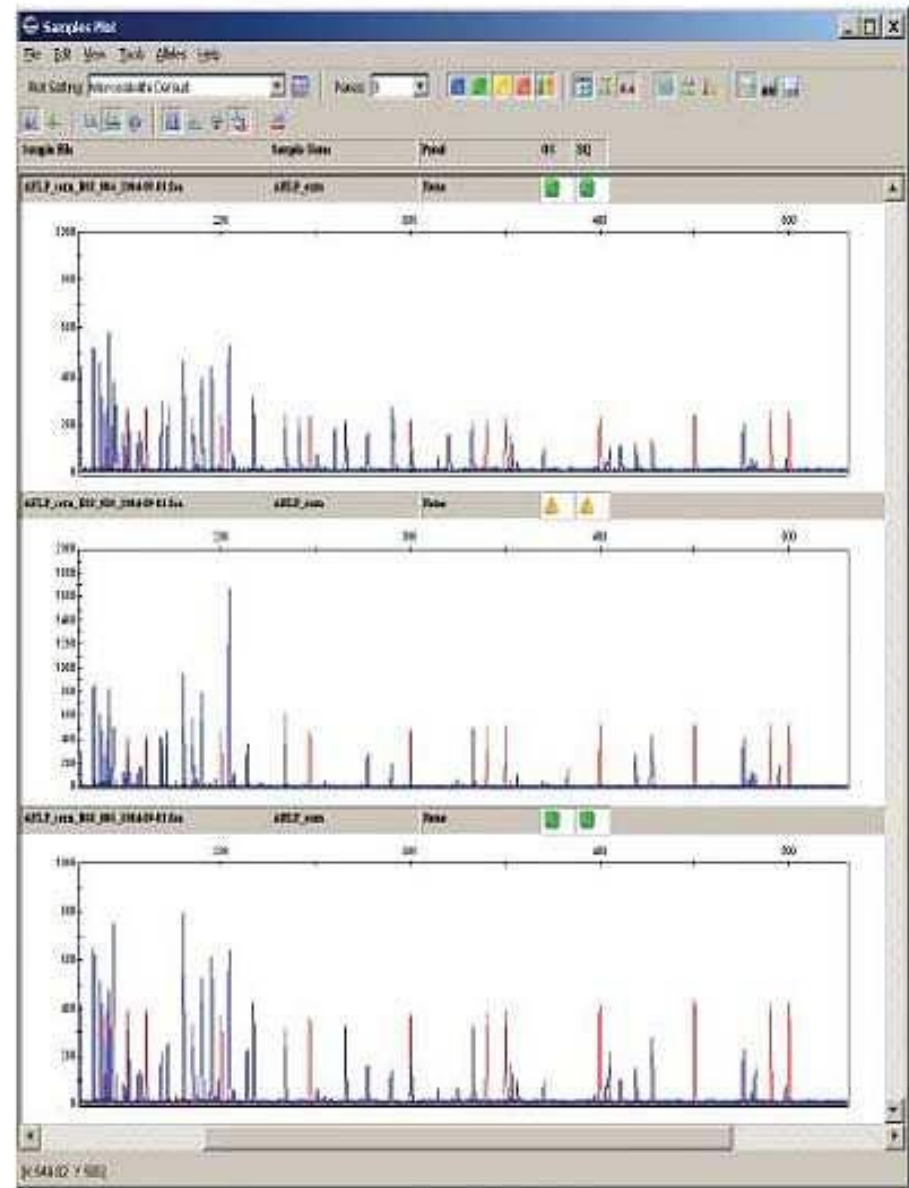
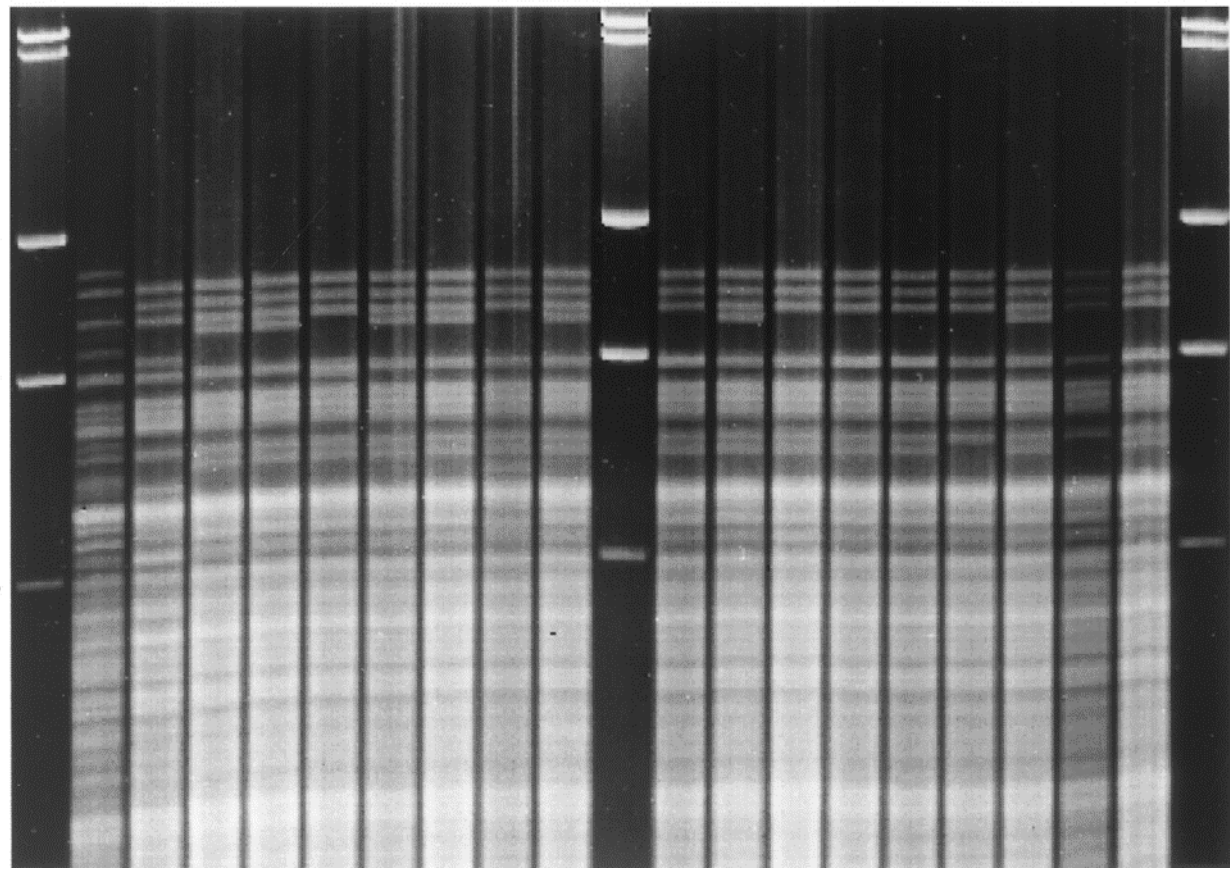
- restriction of genomic DNA and ligation of adaptors (most often performed together) to restricted fragments; (i.e. MseI and EcoRI digestions and MseI-adaptor pair and EcoRI-adaptor pair)
- preselective PCR amplification of a subset of the restricted fragments;
- selective PCR amplification, reducing further fragment number; (i.e EcoRI primers: 5- GACTGCGTACCAATTCXXX where X stands for selective nucleotides)
- electrophoretic separation of amplified DNA fragments (capillary electrophoresis in a Sanger sequencer);
- scoring and interpretation of the data

AFLP procedure



Kb M 1 2 3 4 5 6 7 8 9 M 10 11 12 13 14 15 16 17 18 M

23.1 —
9.4 —
6.6 —
4.4 —



Pulsed-Field Gel Electrophoresis:

This is a widely used technique for analyzing a large amount of chromosomal DNA found in large bacterial chromosomal fragments generated by endonuclease digestion.

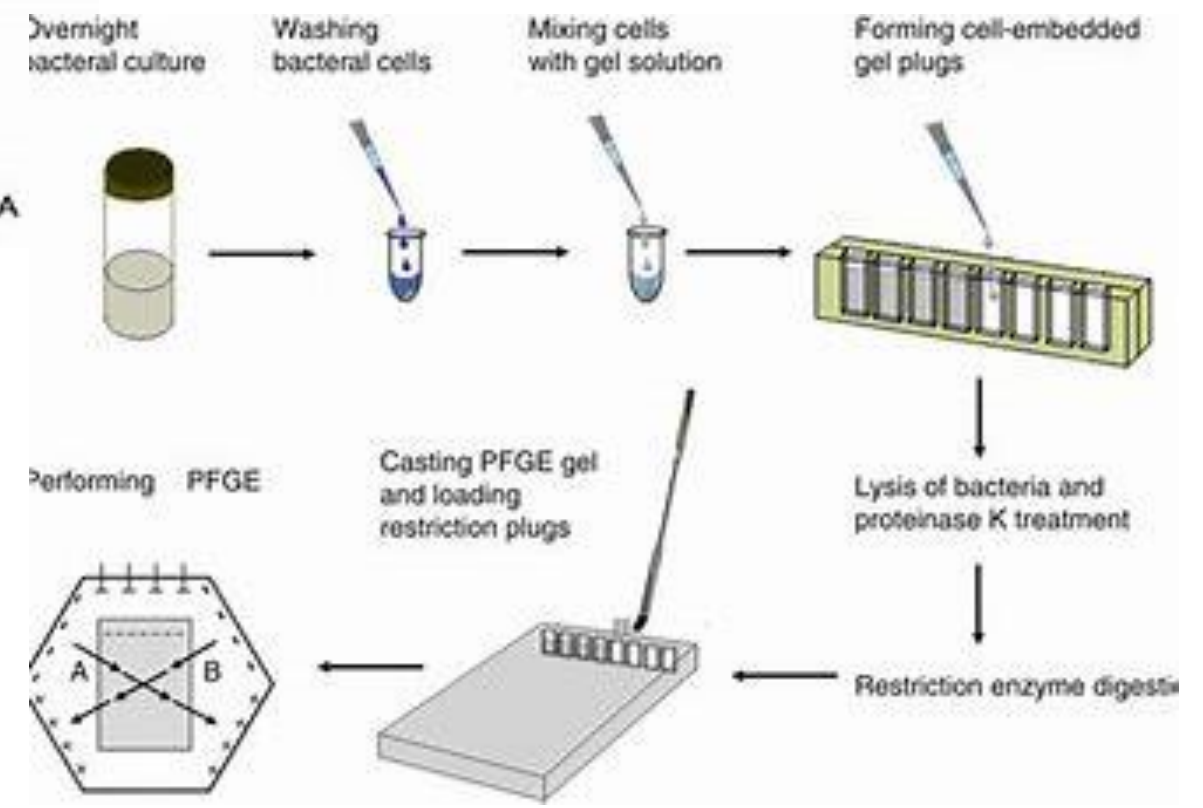
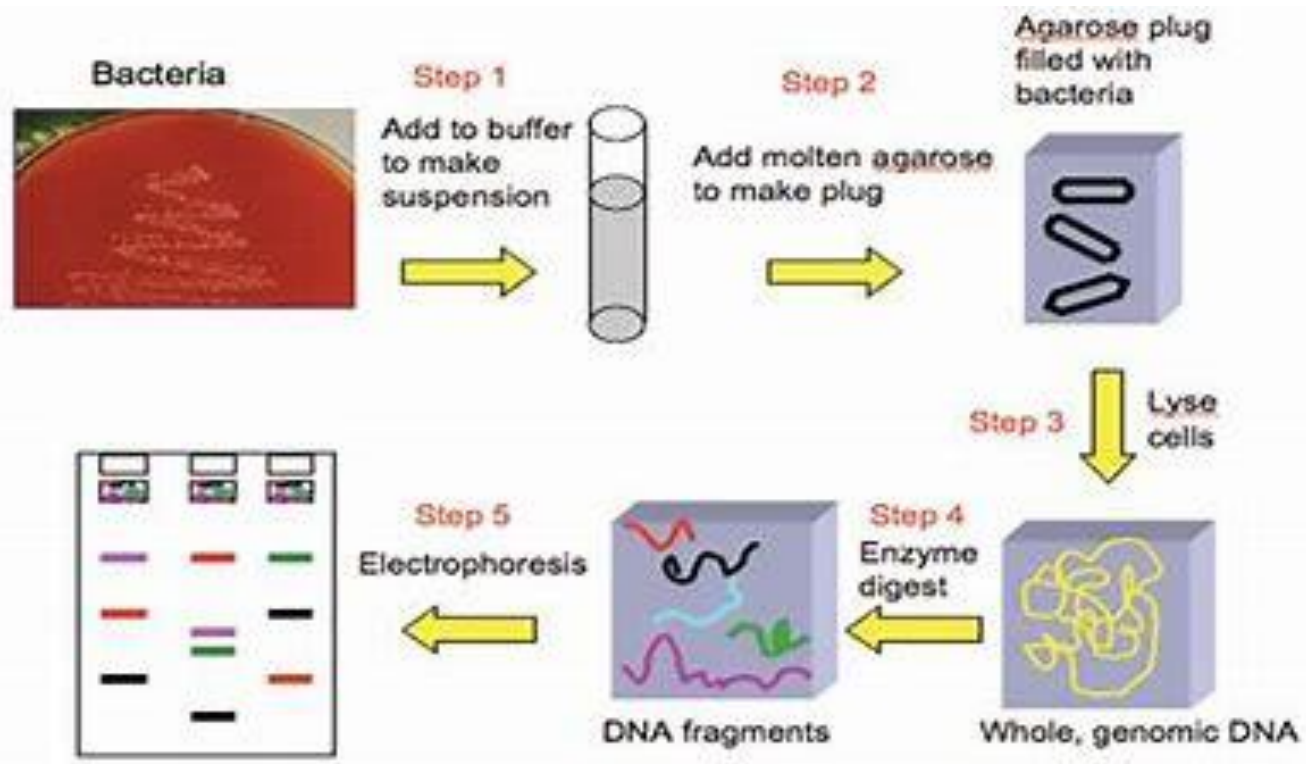
PULSE FIELD GEL ELECTROPHORESIS

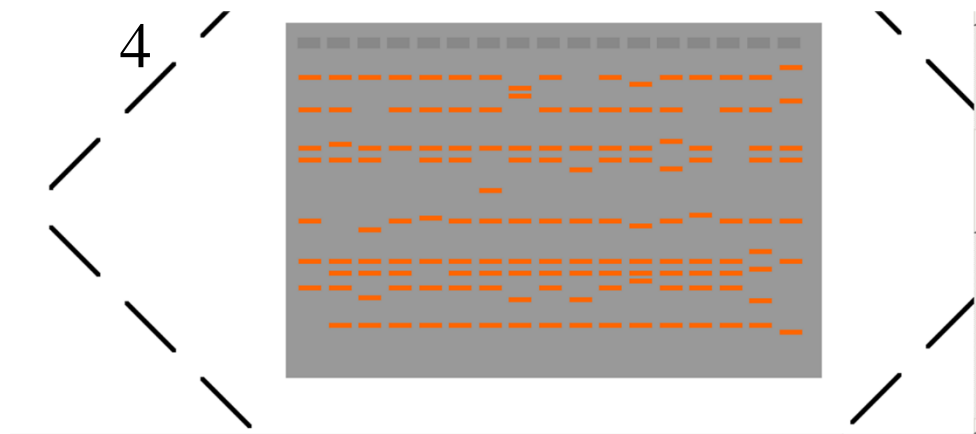
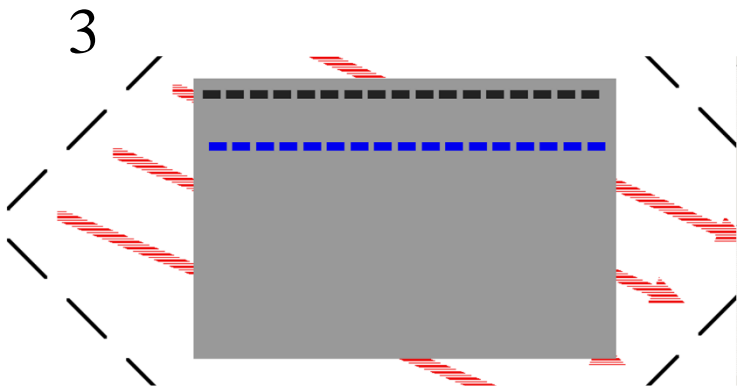
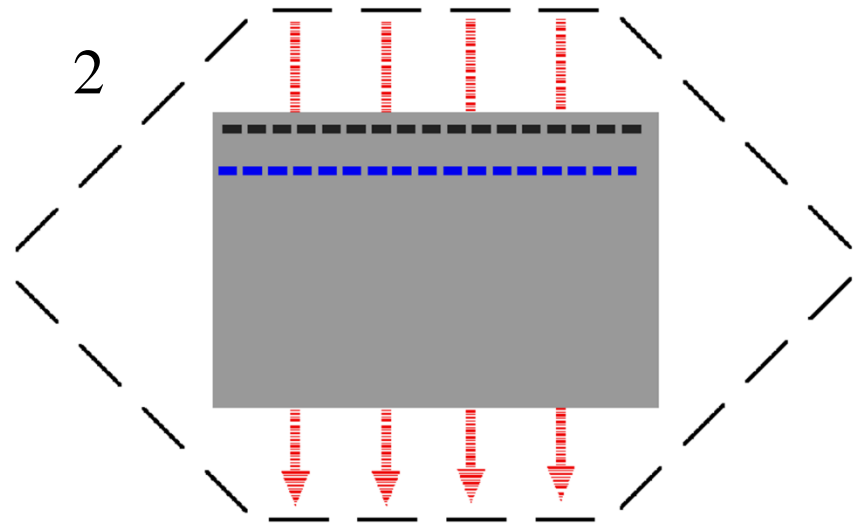
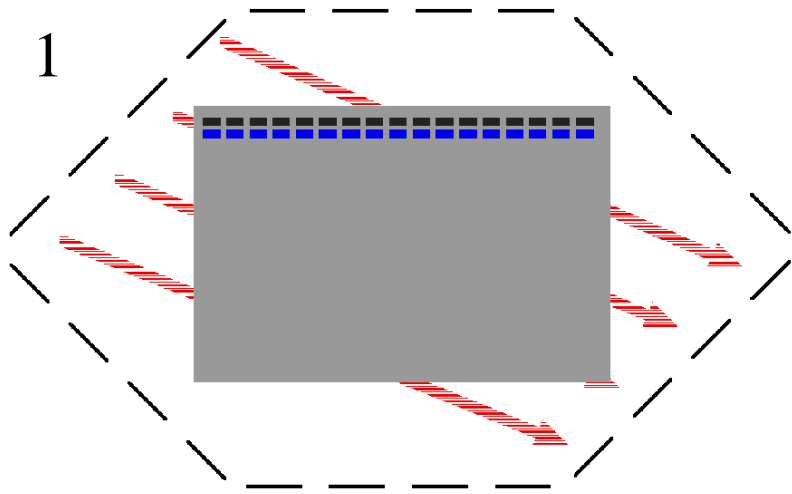
The method consists of an electrophoresis on agarose gel in which two electrical fields with different angles are applied alternately for defined periods of time (e.g. 60s).

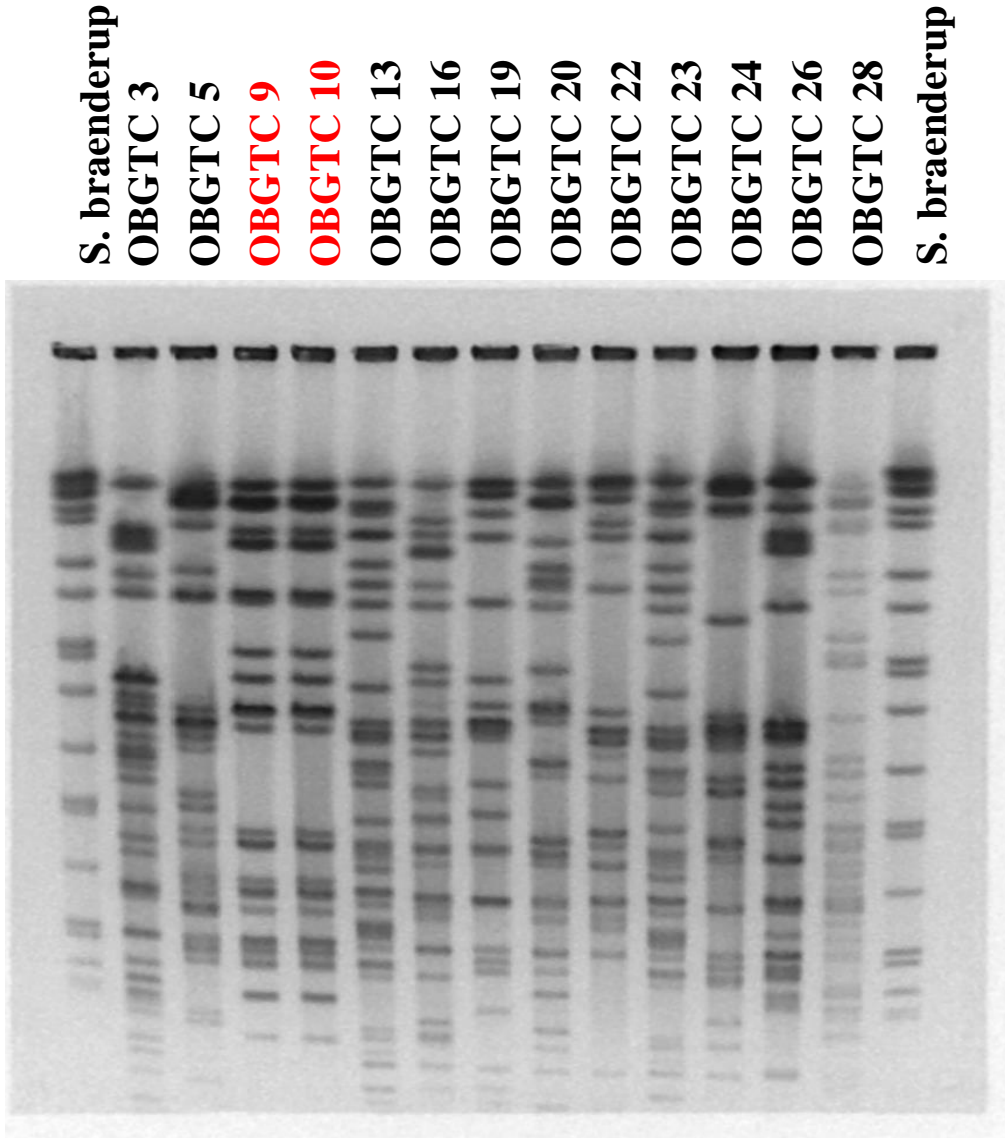
The action of the first electric field induces the movement of DNA fragments along the direction of the field. The interruption of this field and the application of the second causes the molecules to move in the new direction.

Since for a linear long-chain molecule there is a relationship between the conformational change induced by an electric field and the length of the molecule itself, the smaller molecules will realign faster in the new electric field than the larger ones.

In this way, not only the smaller molecules are separated from the larger ones but, thanks to the different re-orientation times typical of larger fragments, also large molecules between them.





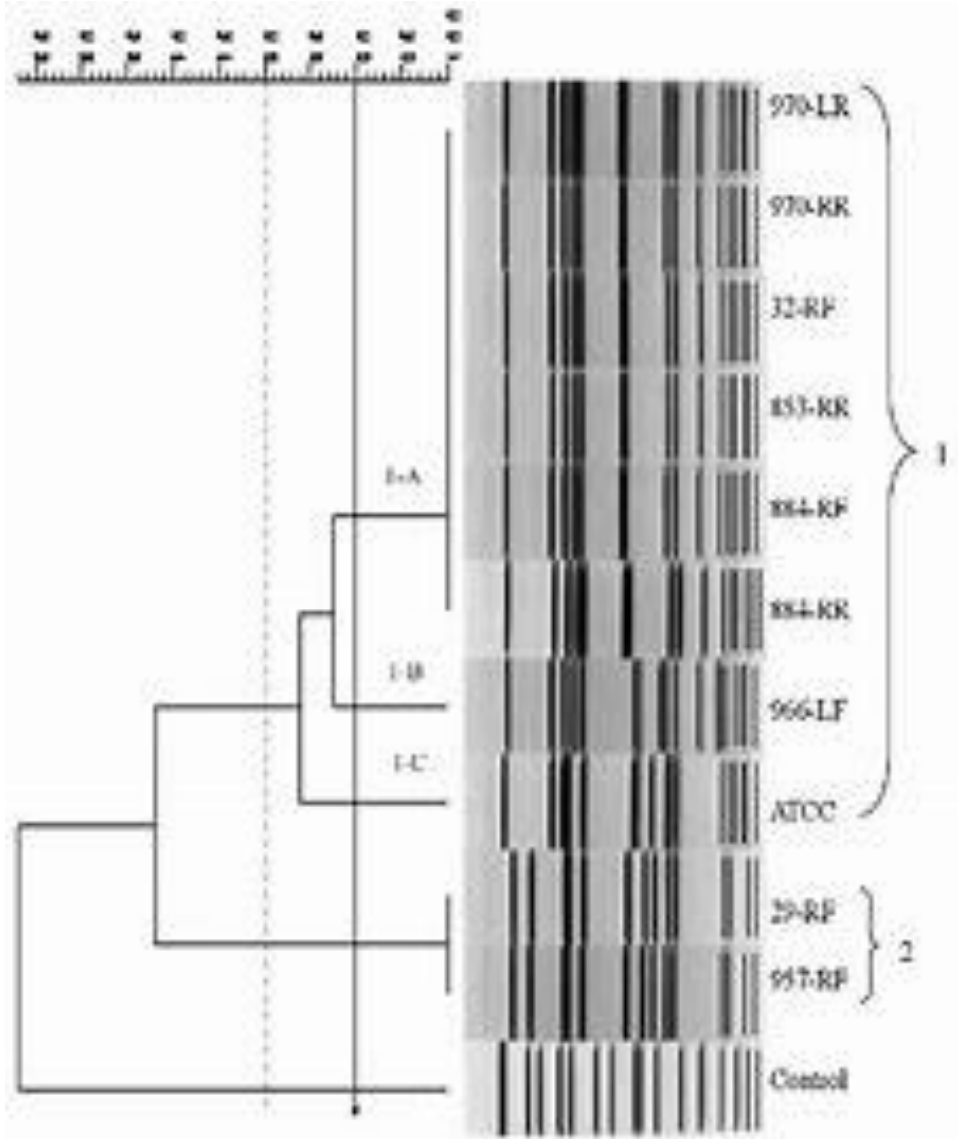


PFGE
XbaI digestion

Band pattern

Pulsotypes

Genomic DNA macrorestriction profiles of *S. maltophilia* produced by PFGE



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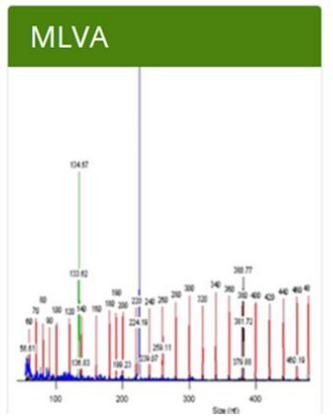
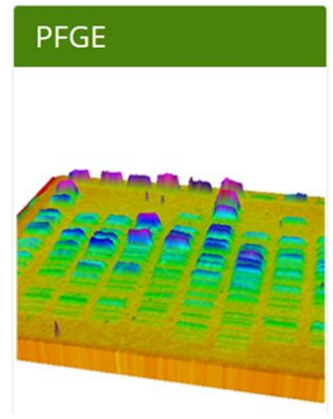
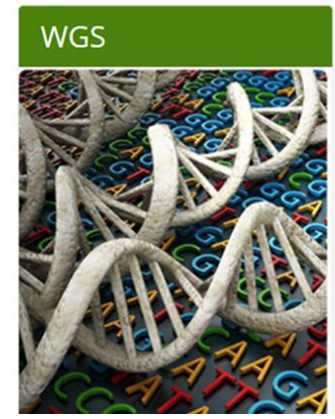
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PulseNet International

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PulseNet Methods

PulseNet uses a variety of methods to subtype: *E. coli* (O157 and other Shiga toxin-producing *E. coli*), *Campylobacter*, *Listeria monocytogenes*, *Salmonella*, *Shigella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Cronobacter* isolates. [Pulsed-field gel electrophoresis \(PFGE\)](#), [multiple locus variable number tandem repeat analysis \(MLVA\)](#), and [whole genome sequencing \(WGS\)](#) are PulseNet's main subtyping (or fingerprinting) tools.



MLVA: Multiple-Locus Variable-number tandem-repeat Analysis

“The MLVA method indexes genetic variation at well defined genomic loci and produces reproducible allelic profiles that can be coded in a simple digital format.

Hence, they represent an attractive alternative to banding profile-based methods such as pulsed-field gel electrophoresis (PFGE), which requires dedicated efforts (e.g.

<http://www.cdc.gov/pulsenet>) in order to produce fingerprinting data that are comparable across laboratories.

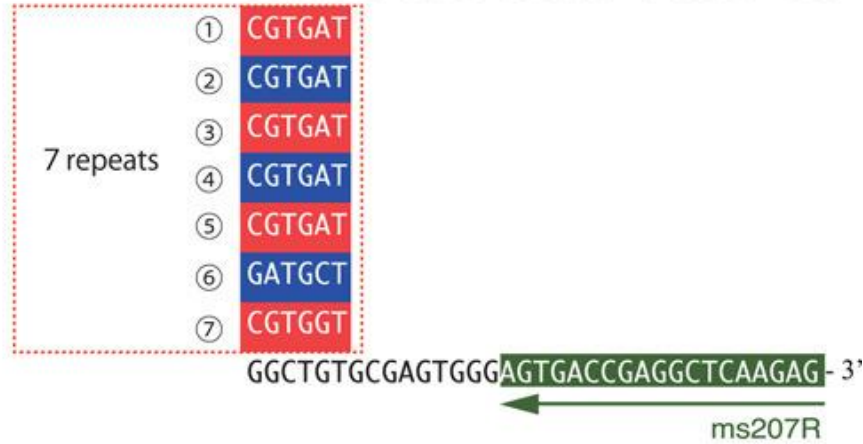
Indeed, to be useful to surveillance networks and for global epidemiology, a genotyping method has to be technically accessible, reproducible and to yield easily portable data. In addition, electronic databases that are made accessible through the Internet can render exchange and comparison of data among laboratories very effective for local, national, and international surveillance.”

Guigon G, Cheval J, Cahuzac R, Brisse S. MLVA-NET – a standardised web database for bacterial genotyping and surveillance. *Euro Surveill.* 2008;13(19):pii=18863.

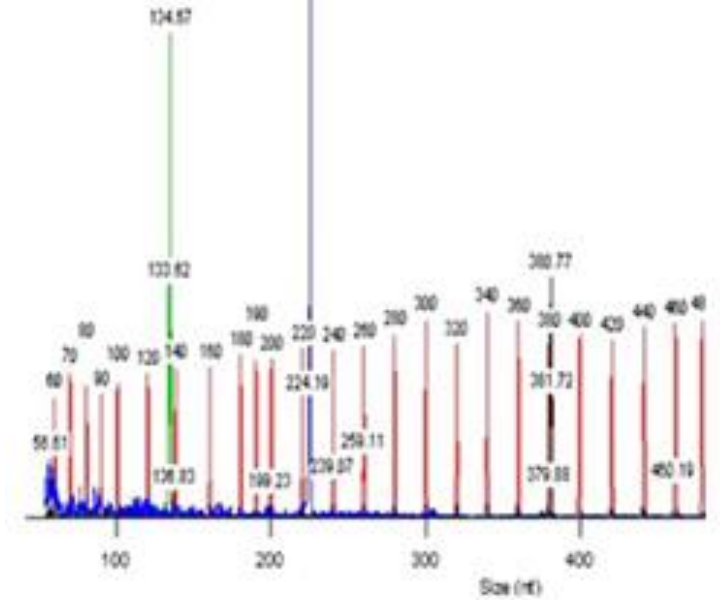
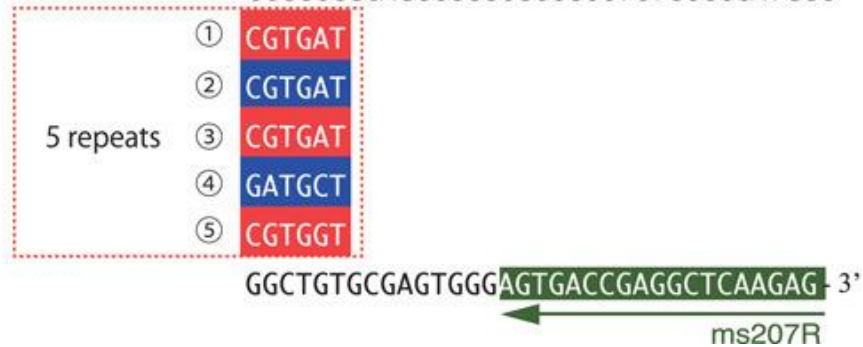
MLVA

ms207: repeat unit size = 6 bp

PAO1: 146 bp 5' **ACGGCGAACAGCACCAGCA**GCGCGGCGACCAGCAG
CCGGCGCCAGGGCGACGCGGCCTGTGCGCCATGGC



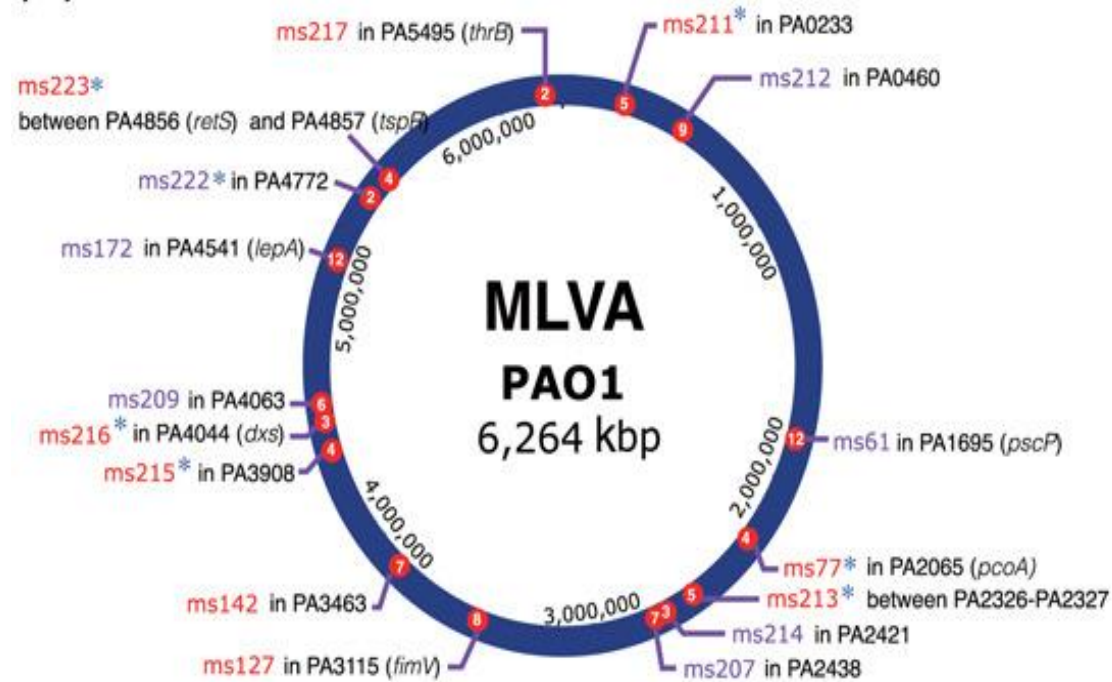
PA14: 135 bp 5' **ACGGCGAACAGCACCAGCA**GCGCGGCGACCAGCAG
CCGGCGCCAGGGCGGCGGCCTGTGCGCCATGGC

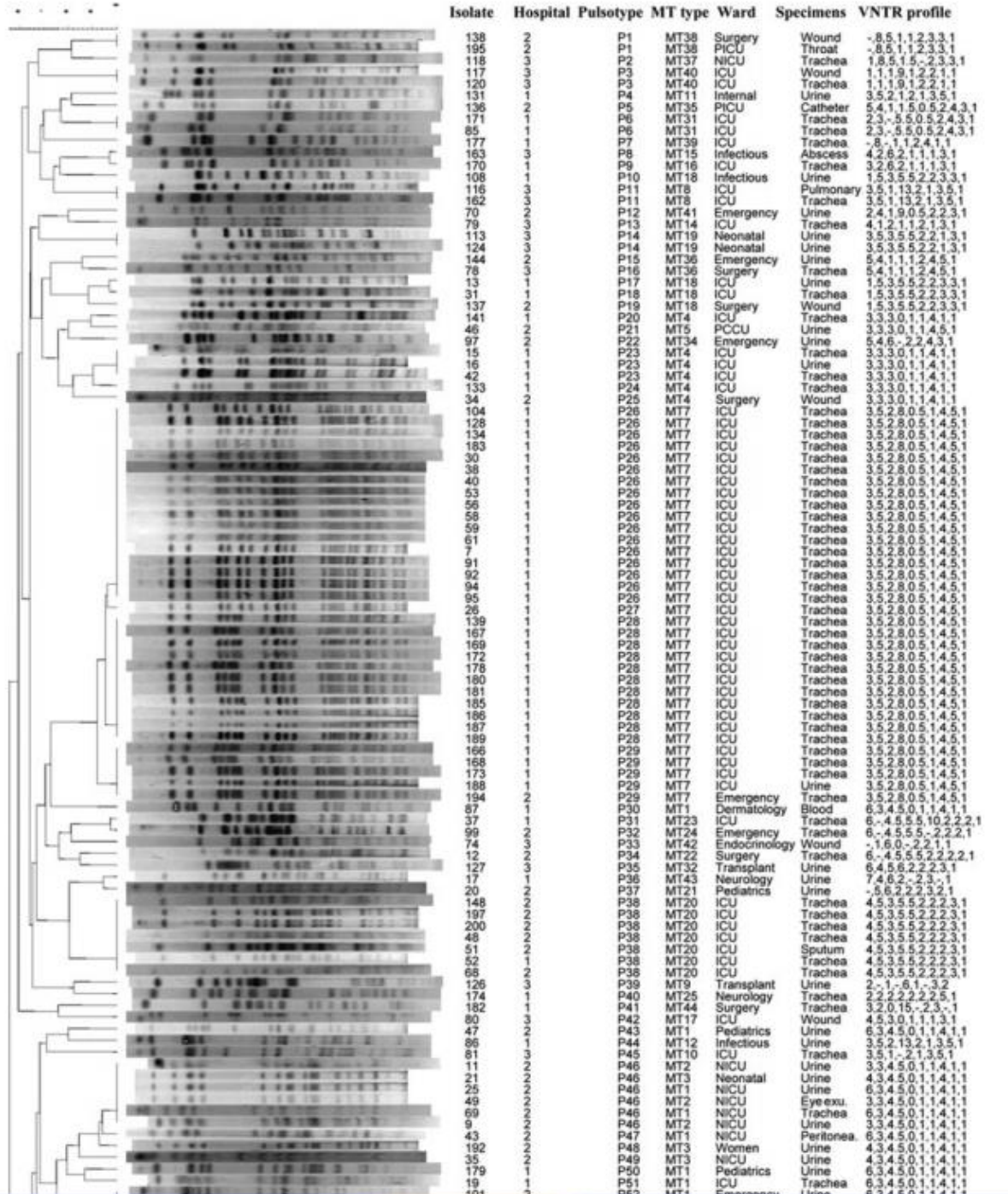


No of repeats

MLVA TYPES

(a)





Derakhshan et al., 2016. Multiple-Locus Variable Number Tandem Repeat Analysis of *Klebsiella pneumoniae* : Comparison with Pulsed-Field Gel Electrophoresis
Microbial Drug Resistance 23(5)

FIGURE 1

MLVA-NET isolates query using the <Search database> menu

Repeat Type Query	Profile Query	Search Database
Browse Database	Database Stats	Isolates Index

**Salmonella enterica subsp. enterica serotype Typhimurium isolates database
Search database**

Combine searches with: Order by:

>
 contains
 =
 =

Show Profiles

Select Fields:

id atb curator
 dataset source submission_date
 strain country date_stamp
 other_name1 year fragment sizes
 other_name2 outbreak allele numbers
 serotype comment rt
 phage_type sourcelab
 pfge sender

Notes: You can vary the number of fields that can be combined by going to the options page.

Guigon G, et al., MLVA-NET – a standardised web database for bacterial genotyping and surveillance. Euro Surveill. 2008;13(19):pii=18863.

FIGURE 2

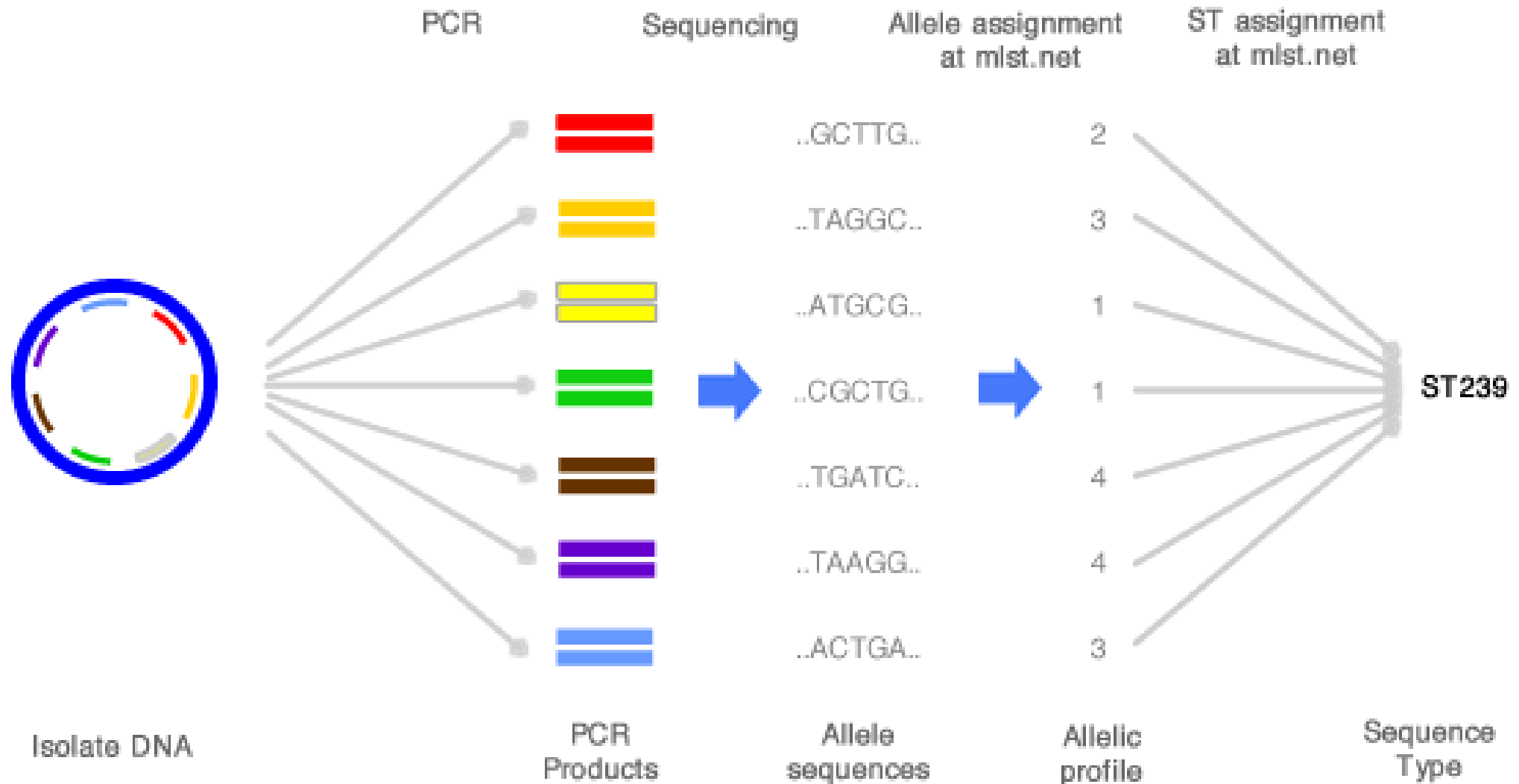
MLVA-NET results page for *Salmonella enterica subsp. enterica serotype Typhimurium* isolates from Norway with allele number 1 for marker STTR9

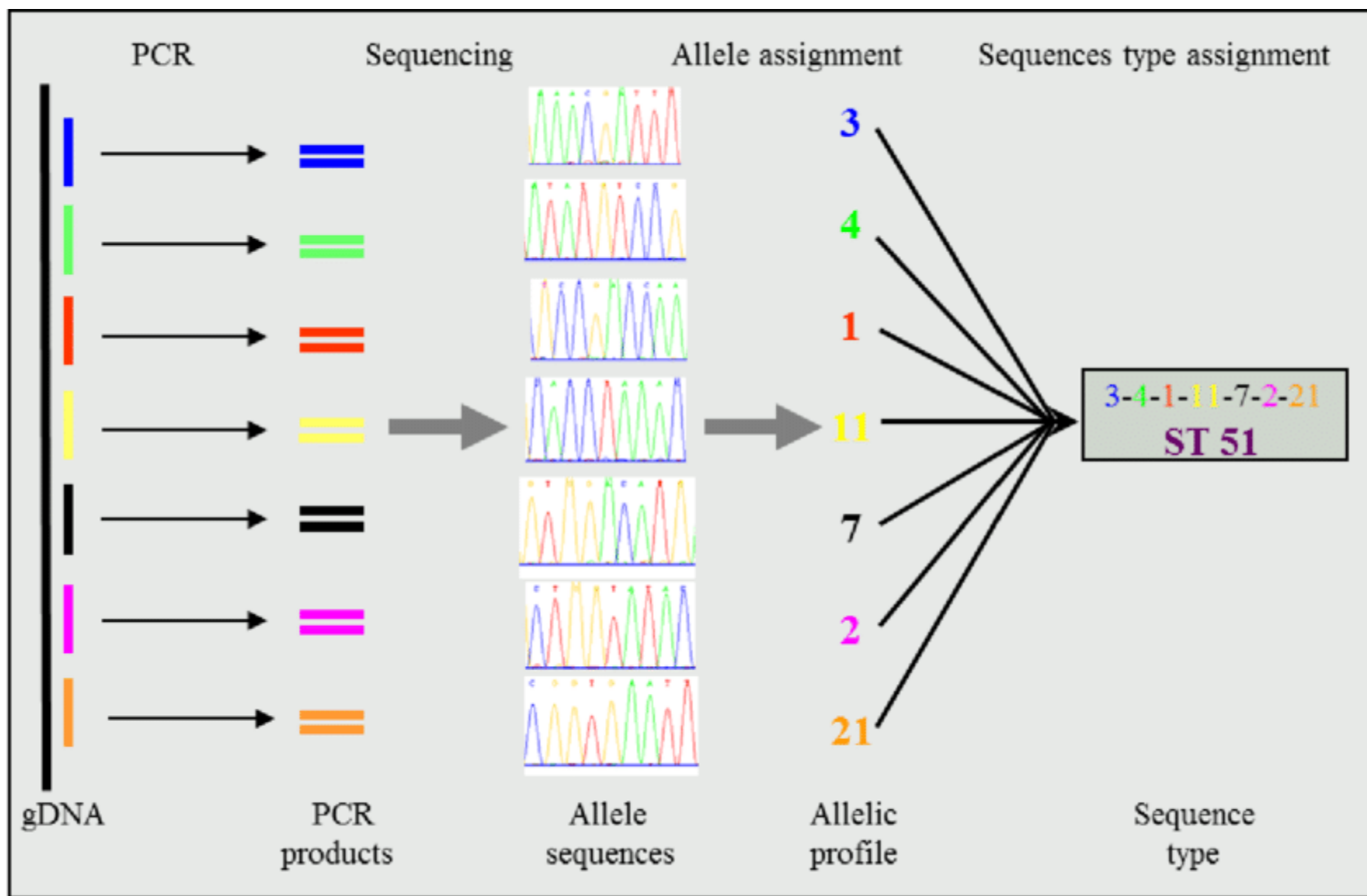
Isolates information									Fragment sizes					Allele numbers					RT
id	strain	serotype	phage_type	source	country	year	sourcelab	date_stamp	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	rt
189	1107-0022			fodder	Norway	2007	NIPH Oslo	2007-11-14	162	227	394	363.00	524.00	1	1	18	14	3	83
369	1107-0768			human	Norway	2007	NIPH Oslo	2007-11-22	162	239	300	362	549	1	3	3	14	4	91
377	1107-0778			bird	Norway	2007	NIPH Oslo	2007-11-22	162	252	394	362	550	1	5	18	14	4	92
380	1107-0793			human	Norway	2007	NIPH Oslo	2007-11-22	162	246	348	350.00	523	1	4	9	19	3	93
423	1107-1051			Environmental	Norway	2007	NIPH Oslo	2007-11-22	162	264	305	344	325	1	7	19	17	8	100
457	1107-1368			human	Norway	2007	NIPH Oslo	2007-11-22	162	306	359	356	523	1	19	11	1	3	105
599	1108-0039			human	Norway	2008	NIPH Norway	2008-01-21	162	300	318	356	524.00	1	10	4	1	3	90
603	1108-0126			human	Norway	2008	NIPH Oslo	2008-02-26	162	246	301	393	523	1	4	3	4	3	134
606	1108-0177			dog	Norway	2008	NIPH Oslo	2008-02-26	161	301	319	357	523	1	10	4	1	3	90
608	1108-0228			human	Norway	2008	NIPH Oslo	2008-02-26	162	300	325	356	524.00	1	10	5	1	3	137

Multilocus Sequence Typing:

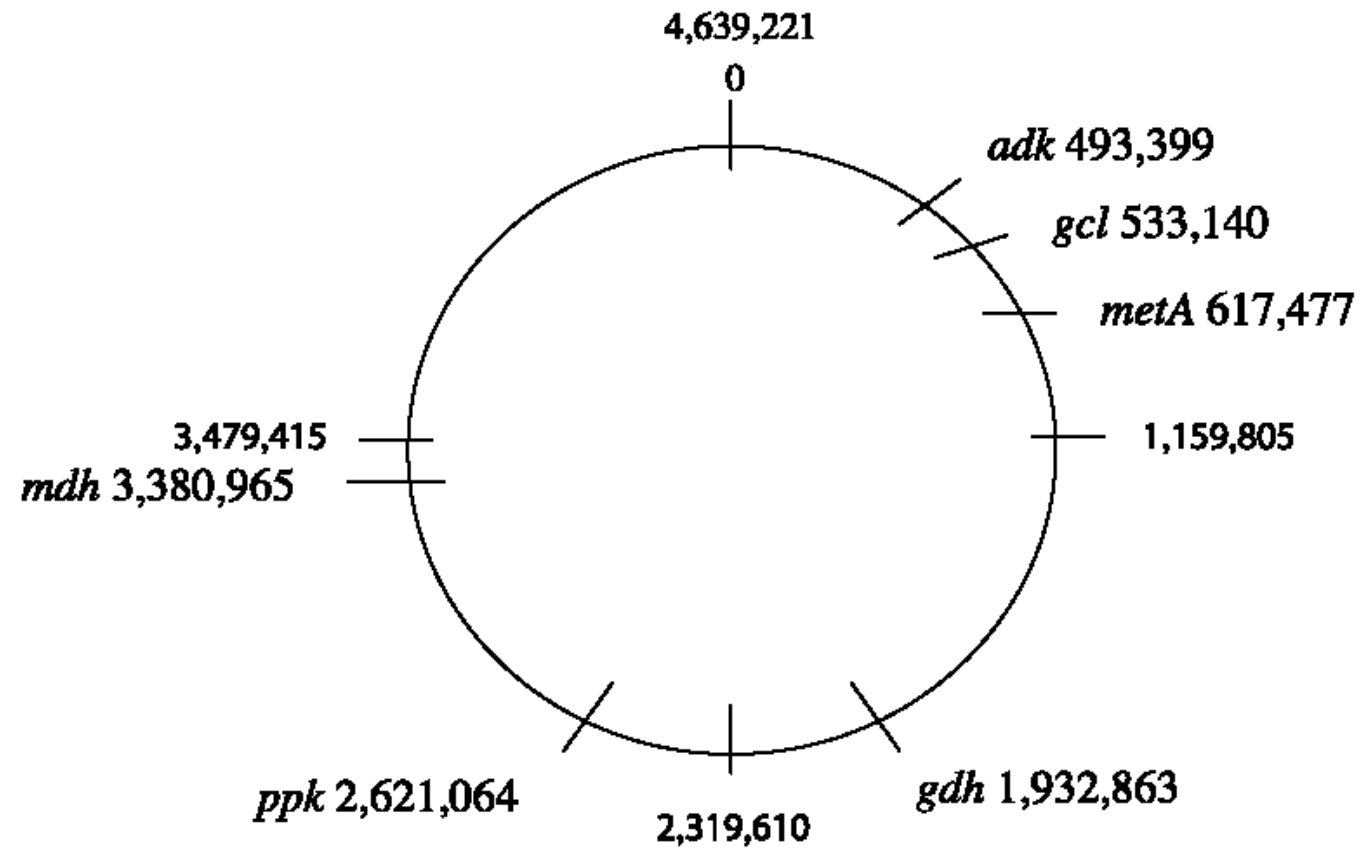
This is a genome-based version of the conventional method of multilocus enzyme electrophoresis. It helps in the typing of various bacterial species by identifying DNA alleles from various organisms.

This method involves PCR amplification and the nucleic acid sequencing of multiple internal fragments of housekeeping genes. The advantages of this method are that the culturing of pathogenic micro-organisms is avoided and that the sequencing data are unambiguous, easy to standardize, and electronically portable.





MultiLocus Sequence Typing: genes along the chromosome



Available Databases

Salmonella

Strains:278376

Assembled

- Legacy:4930
- From NGS:273446
- In Progress:202

Schemes

- Achtman 7 Gene MLST:278334
- cgMLST V2 + HierCC V1:272096
- rMLST:273222
- wgMLST:272534

[Database Home](#)



Escherichia/Shigella

Strains:157082

Assembled

- Legacy:9525
- From NGS:147557
- In Progress:1098

Schemes

- Achtman 7 Gene MLST:156931
- cgMLST V1 + HierCC V1:147448
- rMLST:147458
- wgMLST:147205

[Database Home](#)



Clostridioides

Strains:20223

Assembled

- From NGS:20223
- In Progress:12

Schemes

- cgMLST V1 + HierCC V1:20177
- Griffiths 7 Gene:20222
- rMLST:20222
- wgMLST:20193

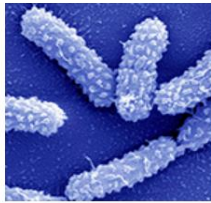
[Database Home](#)



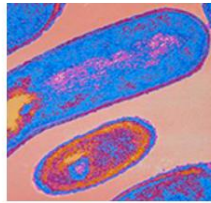
This site hosts databases of multilocus sequence typing (MLST) and whole-genome based typing schemes, which are used for genotyping of bacterial isolates. They provide reference nomenclatures of microbial strains and are mainly intended for molecular epidemiology of pathogens of public health importance, detection of virulence and antimicrobial resistance genes, and for population biology research. This site is powered by the [BIGSdb](#) software.

Databases hosted on this site

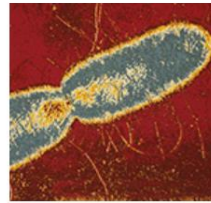
Klebsiella pneumoniae



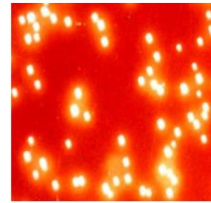
Listeria monocytogenes



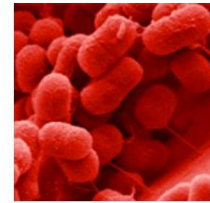
Escherichia coli



Bordetella



Acinetobacter baumannii



Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates



A collection of open-access, curated databases that integrate population sequence data with provenance and phenotype information for over 100 different microbial species and genera.

23,006,368
ALLELES

809,528
ISOLATES

566,861
GENOMES



Organisms search

Organisms

Most popular



Campylobacter jejuni/coli



Haemophilus influenzae



Neisseria spp.



Staphylococcus aureus

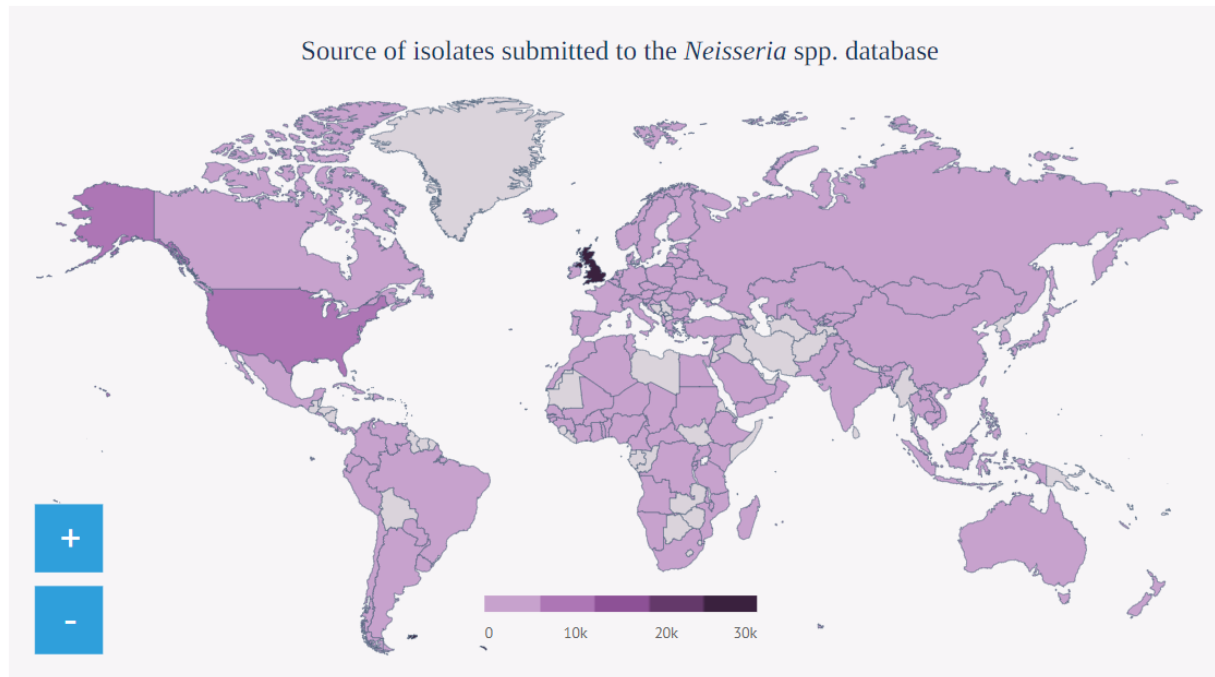


Streptococcus agalactiae



Streptococcus pneumoniae

Neisseria spp.



↑ SUBMIT

- Curation and management
- Target genes/antigens
 - MLST
 - PorA variable regions
 - FetA variable region
 - *porB*
 - fHbp
 - NHBA
 - NadA
- Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR)

The preferred citation for this website is:

Jolley *et al.* *Wellcome Open Res* 2018, **3**:124 [version 1; referees: 2 approved]

MLST genes

abcZ (putative ABC transporter)

adk (adenylate kinase)

aroE (shikimate dehydrogenase)

fumC (fumarate hydratase)

gdh (glucose-6-phosphate dehydrogenase)

pdhC (pyruvate dehydrogenase subunit)

pgm (phosphoglucomutase)