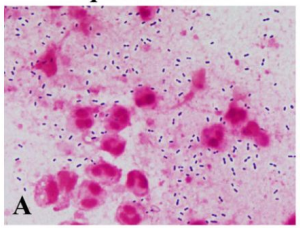
MGMA2024

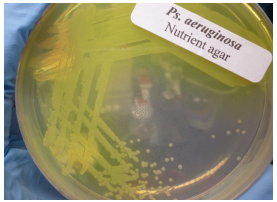
Lecture 11. Serotyping, genotyping, phenotyping microbes

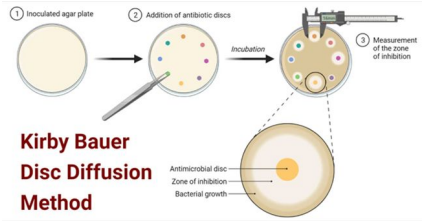
Section 2. Introducing Methods for Subtyping Microbes

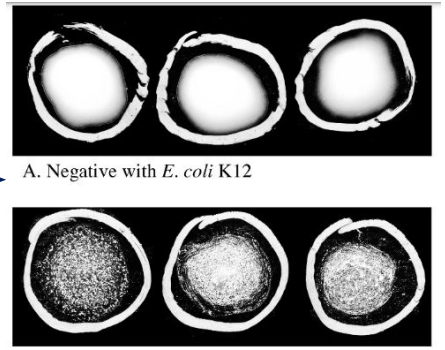
SUBTYPING METHODS

S. pneumoniae





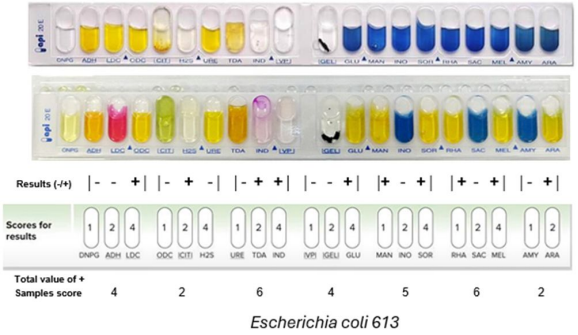




A. Negative with *E. coli* K12

B. Positive with *E. coli* O26

FIGURE 1. *Typical latex agglutination results with latex anti-O26 STEC.*

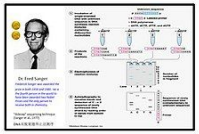


Escherichia coli 613

Culture-positive samples → **Phenotyping and Serotyping**

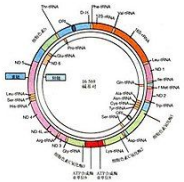
Culture-negative samples → **Methods?**

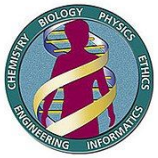
A brief history of Next Generation Sequencing (NGS)



1977 Sanger method

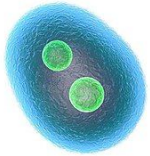
1981 Human mitochondrial genome sequence





1990 Human Genome Project

1995 Complete cell genome



1996 Complete eukaryotic genome

2001 Complete the Human Genome Project

2005 Second generation sequencer：454 GS20

2007 Second generation sequencer：Genetic Analyzer 2

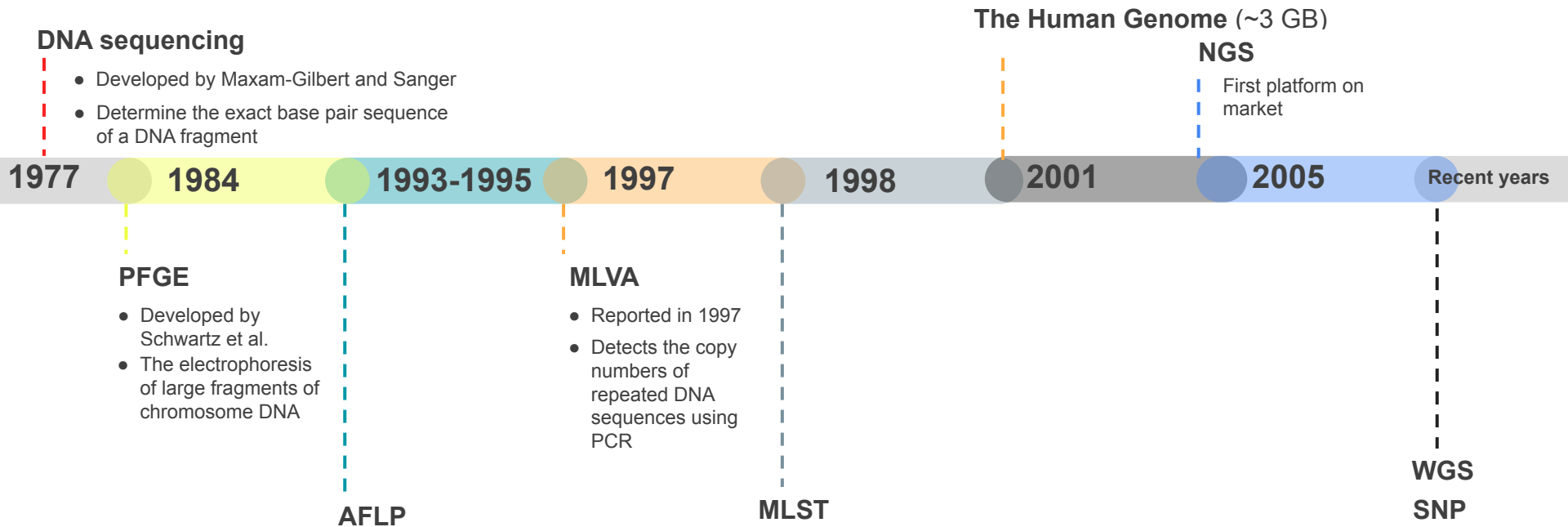
2008 Research Human Microbiome Project

2011 Third generation sequencer：PacBio RS

2014 Nanospace sequencing

2019 The third stage Human microbiome project

Timeline of the development of molecular subtyping methods



Molecular subtyping methods

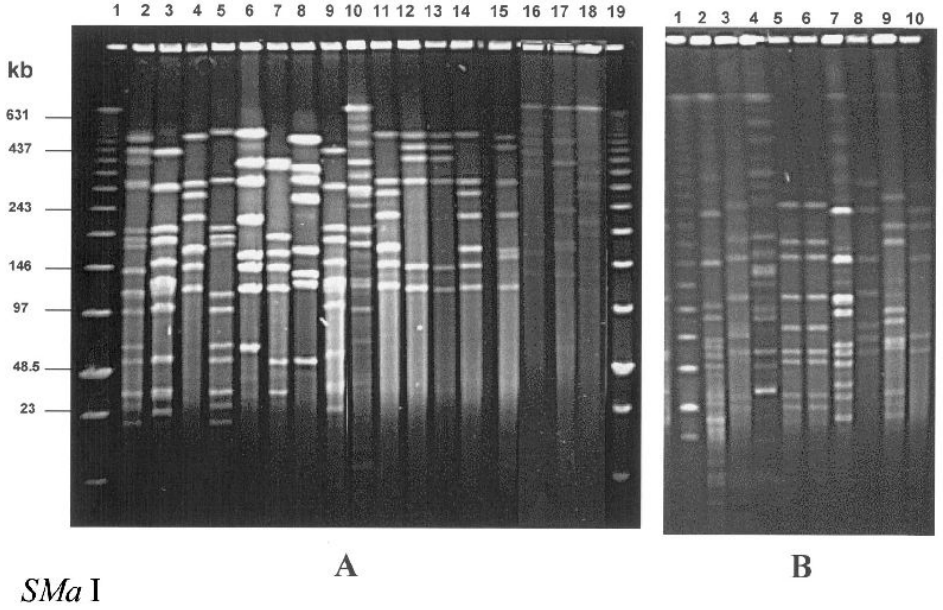
Band –base methods
(Based on comparing variation in DNA banding pattern on gels)

- **Restriction digestion-based method**
  - **P**ulsed **F**ield **G**el **E**lectrophoresis (PFGE)
- **Amplification-based method**
  - **A**mplifiled **F**ragment **L**ength **P**olymorphisms (AFLP)
  - **M**ultiple- **L**ocus **V**ariable number tandem repeat **A**nalysis (MLVA)

Sequence-Based methods
(Based on polymorphism of DNA sequences)

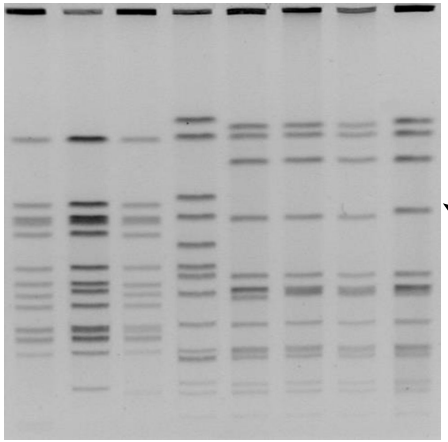
- **M**ulti **L**ocus **S**equence **T**yping (MLST)
  - Traditional MLST
  - cg MLST
  - wg MLST
- **W**hole **G**enome **S**equence (WGS)
- **S**ingle-**N**ucleotide **P**olymorphism (SNP)

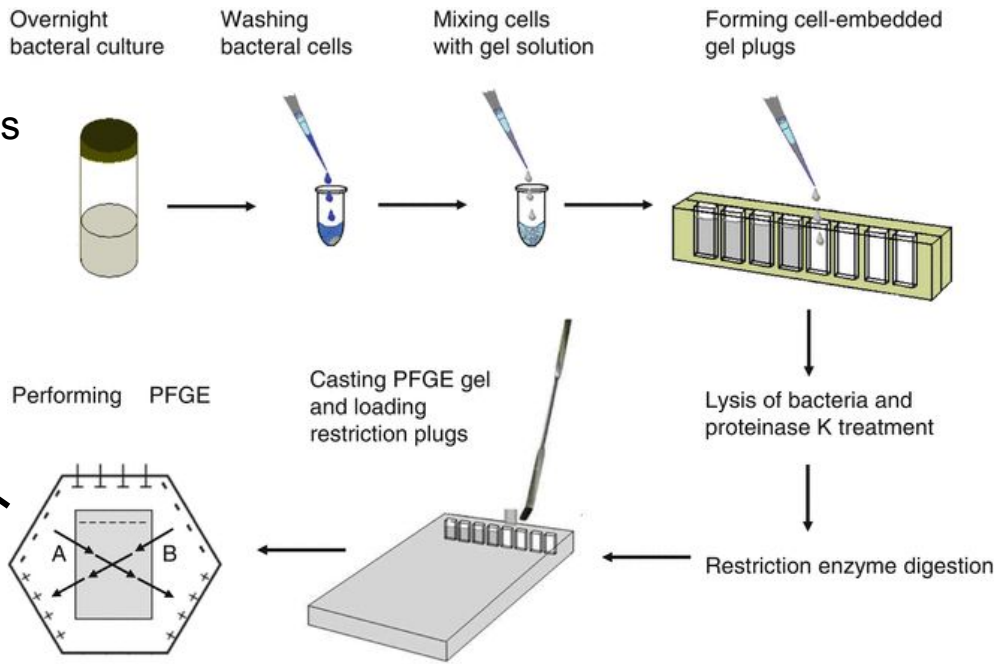
BAND – BASE METHODS



Band –base methods

- **P**ulse **F**ield **G**el **E**lectrophoresis (**PFGE**)
  - The electrophoresis of large fragments (10 Mb) of chromosome DNA
  - The direction of the electric field is constantly changed
  - The lysis of bacterial cells and cut the chromosomal DNA using restriction enzymes





https://doi.org/10.1007/978-1-4939-2599-5_14

Band –base methods

❖ Pulse Field Gel Electrophoresis (PFGE)

❑ Application

- Standard genetic typing method
- Epidemiological studies *(e.g. S. aureus, N. meningitidis, V. cholerae, M. tuberculosis)*

❑ Requirement

- Instruments (CHEF/FIGE…)
- The lysing methods
- Selection restriction enzymes
- Protocol for electrophoresis

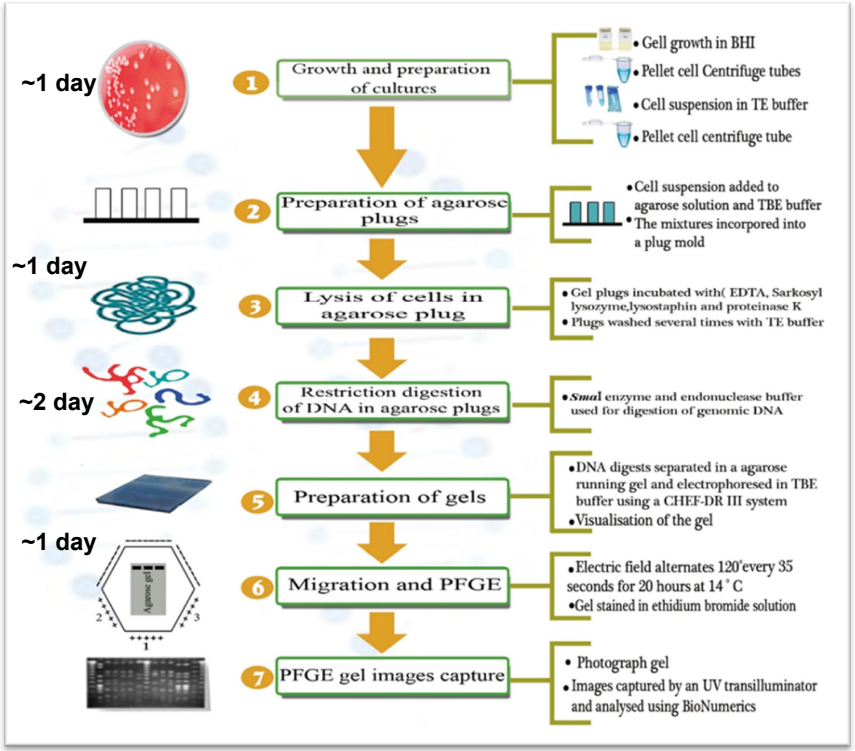
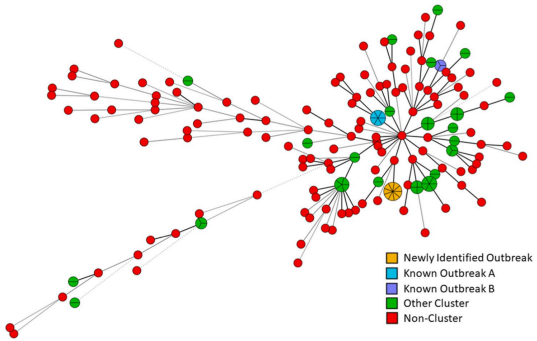


Figure 1. PFGE process for the subtyping of *S. aureus* strains**

https://doi.org/10.7860%2FJCDR%2F2016%2F15718.7043
https://doi.org/10.3390/pathogens12070966

Band –base methods

- **M**ultiple-**L**ocus **V**ariable number tandem repeat **A**nalysis (**MLVA**)
  - From data genomic sequencing
  - Individual strains often carry the same elements with different copy numbers
  - Display all MLVA profiles, MLVA clusters and Minimum spanning tree



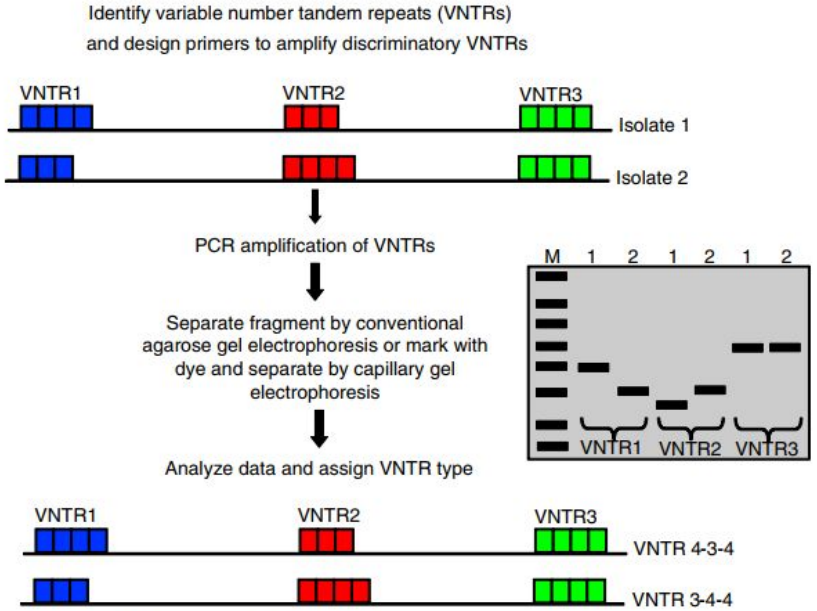


Figure 3 Multiple-locus variable-number tandem repeat analysis (MLVA).

Current Research in Parasitology & Vector-Borne Diseases 4 (2023) 100151

https://doi.org/10.1016/B978-0-12-384731-7.00066-0

Band –base methods

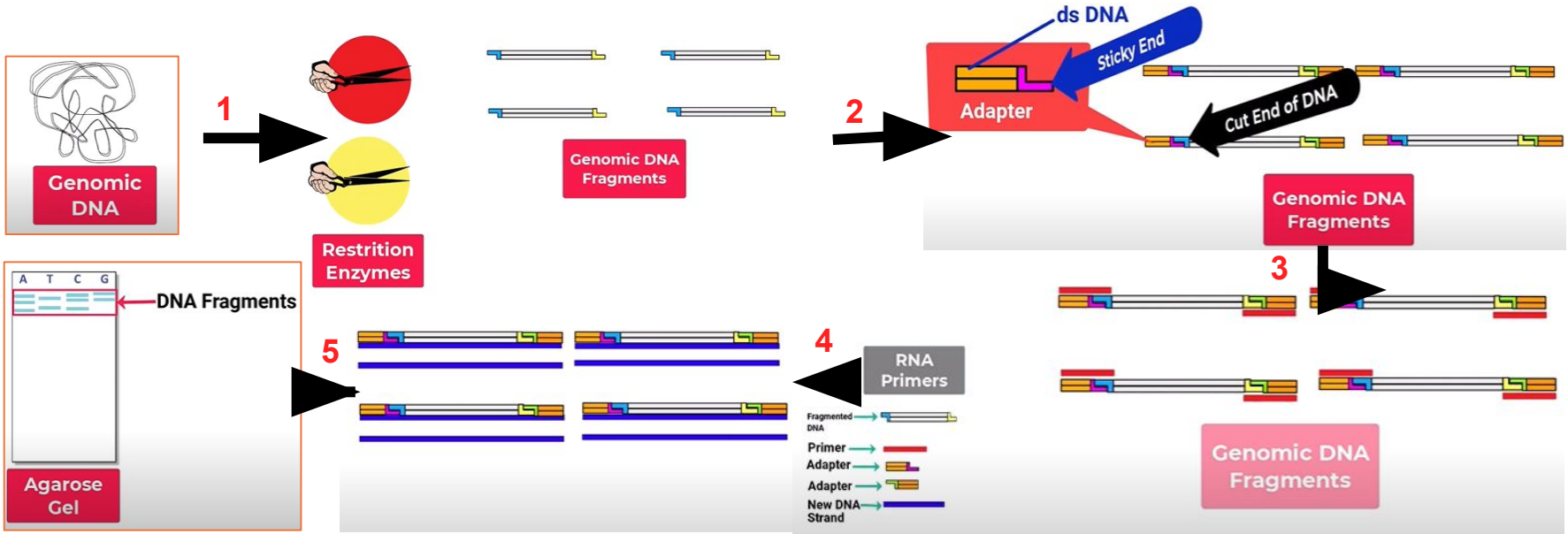
- **M**ultiple-**L**ocus **V**ariable number tandem repeat **A**nalysis (**MLVA**)

  - **Application**
    - The most recent analysis of multiple prokaryotic
      e.g. E. coli O157, Mycoplasma pneumoniae, Cryptosporidium parvum*
    - The epidemiological surveillance, inferring linkage and the investigation of outbreaks
  - **Requirement**
    - Data from genomic sequencing projects
    - Design multiplex PCR primers (loci containing tandem repeats)
    - Assessed on agarose gel/capillary electrophoresis
    - BioNumerics software

*doi:10.1371/journal.pone.0064607

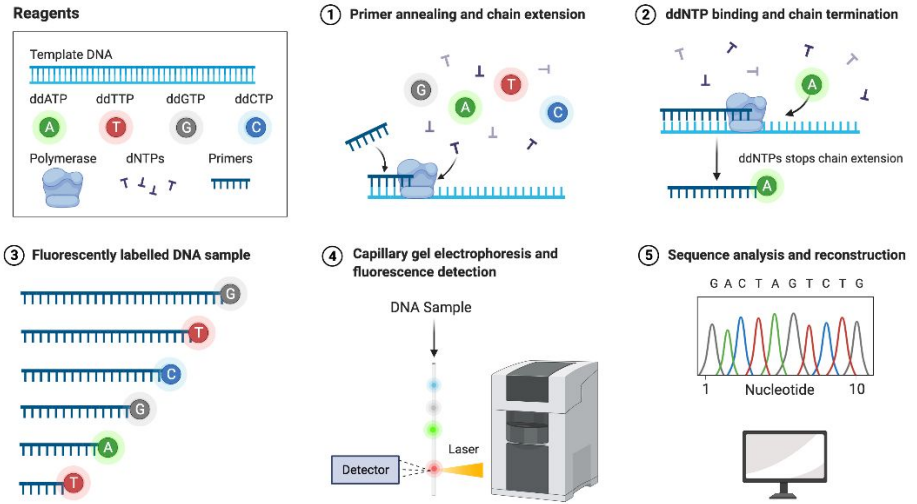
Band –base methods

- **A**mplified **F**ragment **L**ength **P**olymorphism (**AFLP**)



https://www.youtube.com/watch?app=desktop&v=kuRuY25z9TY

SEQUENCE-BASED METHODS

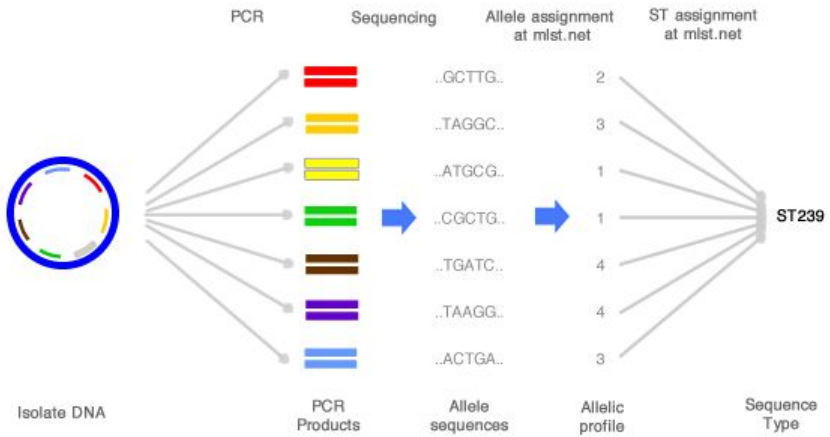


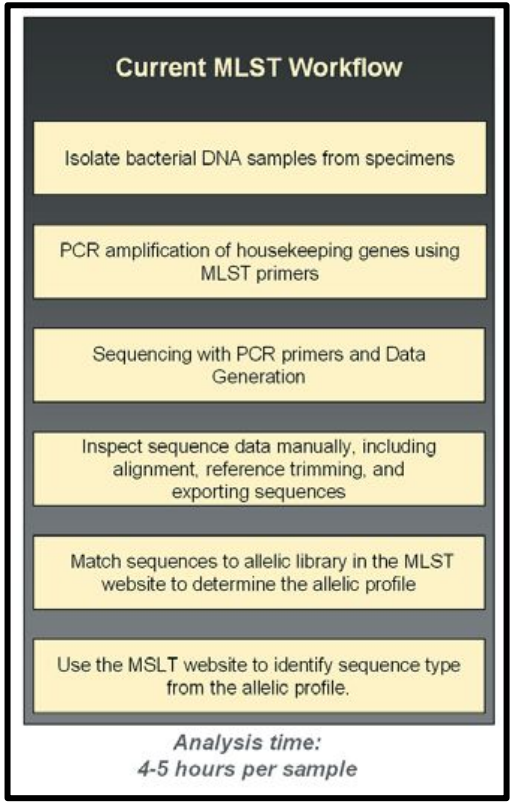
Sequence-based methods

- **M**ulti-**L**ocus **S**equence **T**yping (**MLST**)

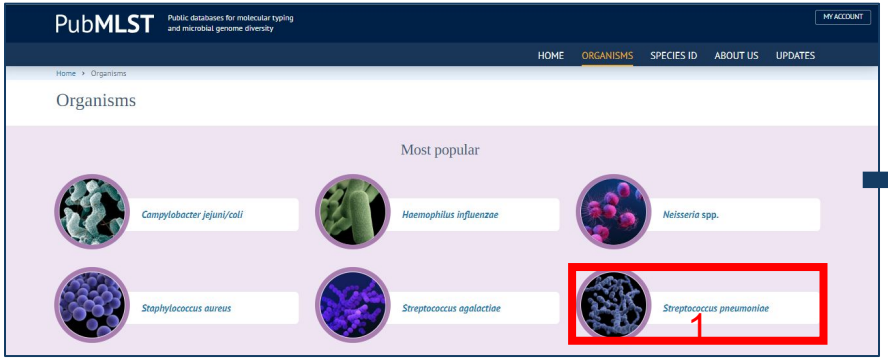
Traditional MLST

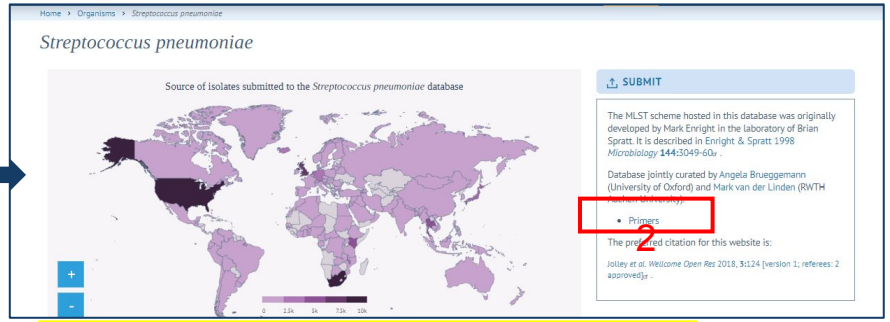
- Using fragments of 7 house-keeping genes (loci)
- 450-500 bp each gene
- Define the allelic profile or sequence types
- Comparisons result with a centralized database (PubMLST;*pubmlst.org*)





Sequence-based methods





Primers and PCR conditions for MLST of *S. pneumoniae*

The pneumococcal MLST scheme uses internal fragments of the following seven house-keeping genes:

- aroE (shikimate dehydrogenase)
- gdh (glucose-6-phosphate dehydrogenase)
- gki (glucose kinase)
- recP (transketolase)
- spi (signal peptidase I)
- xpt (xanthine phosphoribosyltransferase)
- ddl (D-alanine-D-alanine ligase)

The primer pairs used for the PCR amplification of internal fragments of these genes are:

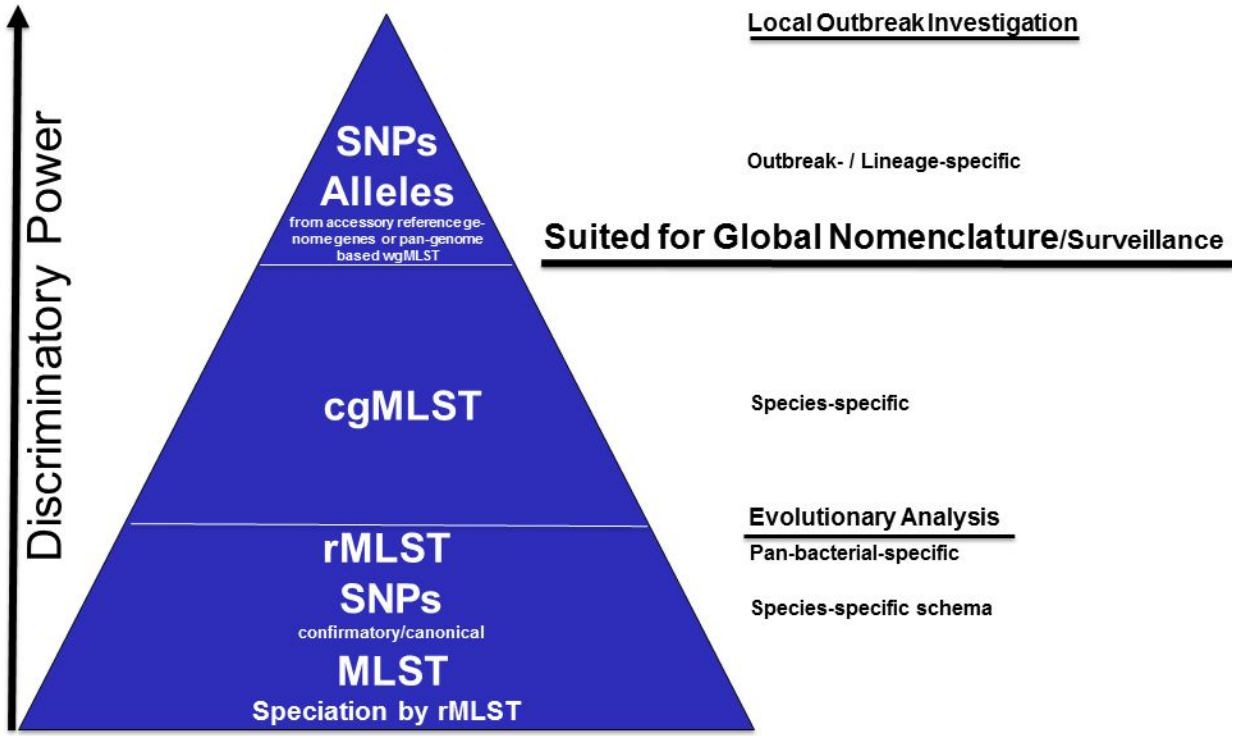
```
aroE-up, 5'-GCC TTT GAG GCG ACA GC
aroE-dn, 5'-TGC AGT TCA (G/A)AA ACA T(A/T)T TCT AA

gdh-up, 5'-ATG GAC AAA CCA GC(G/A/T/C) AG(C/T) TT
gdh-dn, 5'-GCT TGA GGT CCC AT(G/A) CT(G/A/T/C) CC

gki-up, 5'-GGC ATT GGA ATG GGA TCA CC
gki-dn, 5'-TCT CCC GCA GCT GAC AC

recP-up, 5'-GCC AAC TCA GGT CAT CCA GG
recP-dn, 5'- TGC AAC CGT AGC ATT GTA AC

spi-up, 5'-TTA TTC CTC CTG ATT CTG TC
spi-dn, 5'-GTG ATT GGC CAG AAG CGG AA

xpt-up, 5'-TTA TTA GAA GAG CGC ATC CT
xpt-dn, 5'-AGA TCT GCC TCC TTA AAT AC.

ddl-up, 5'-TGC (C/T)CA AGT TCC TTA TGT GG
ddl-dn, 5'-CAC TGG GT(G/A) AAA CC(A/T) GGC AT
```

PCR amplification is carried out on chromosomal DNA using an extension time of 30 seconds, and an annealing temperature of 50 °C, with Taq polymerase. As the same primers are used for amplification and sequencing, it is important that only a single DNA fragment is amplified in the initial PCR. This may involve some optimisation of the annealing temperature.

The following housekeeping genes and primers for MLST

https://pubmlst.org/organisms

Sequence-based methods



Molecular Typing Methods

Typing Method	Basis Features	Public Database Link
PFGE	• Commonly used for epidemiological investigations of foodborne diseases and others	• www.pulsenetinternational.org
AFLP	• Analyses a subset of DNA regions of restriction enzyme-digested bacterial genome	
MLVA	• Determines the number of tandem repeats in multiple loci; capable of detecting genetic differences between strains of highly homogeneous species	• http://www.pasteur.fr/mlva • http://minisatellites.upsud.fr/MLVAnet • http://www.pulsenetinternational.org/protocols/Pages/mlva.aspx
MLST	• Standard strain typing method including six to eight loci; typing scheme has been developed for many bacterial species	• http://pubmlst.org • http://www.mlst.net
WGS	• Detects genetic variations at the genome level and provides higher resolution than other common typing methods	• http://www.ncbi.nlm.nih.gov/genome/ • http://img.jgi.doe.gov
SNP	• Targeting polymorphic sequences of the whole or partial genome sequences; valuable for tracking the spread of monomorphic pathogens	

Molecular Typing Methods

Features of molecular strain typing methods for bacterial organisms

Method	Type of markers used for differentiation	Discriminatory power	Reproducibility	Bioinformatic knowledge needed	Cost
Pulsed-field gel electrophoresis (PFGE)	Number of bands depending on restriction enzyme	•	••	•	••
Multilocus sequence typing (MLST)	7–8 housekeeping genes	••	••••[a]	••	••
Core genome MLST (cgMLST)	Hundreds to thousands of core genes	•••	••••[a]	•••	•••
Whole genome MLST (wgMLST)	Hundreds to thousands of core plus accessory genes	•••	••••	•••	•••
Reference-based single nucleotide polymorphism (SNP) calling	Depends on organism of interest plus reference choice	••••	•••	••••	••••
Reference-agnostic/k-mer based SNP calling	Depends on organism of interest	••••	••••	••••	••••

• low, •• medium, ••• high, •••• very high.

[a]Generally high, but depends on organism of interest and chosen reference.

Curr Opin Infect Dis. 2021 August 01; 34(4): 339–345. doi:10.1097/QCO.0000000000000743.