Finding Your Perfect Match – Evolving Technologies for Bacterial Strain Typing

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Scientist, ATCC

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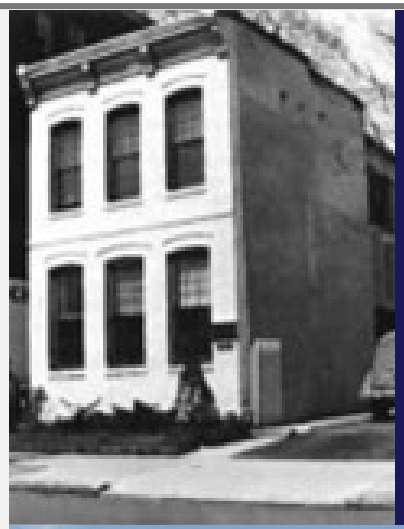
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA and an R&D and Services center in Gaithersburg, MD (225,000 sq. ft. total)
- World's largest and most diverse biological materials resource center
  - 5,000 cell biology products
  - 80,000 microorganisms
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Talented team of 450+ employees; over one-third with advanced degrees



Established partner to global researchers and scientists

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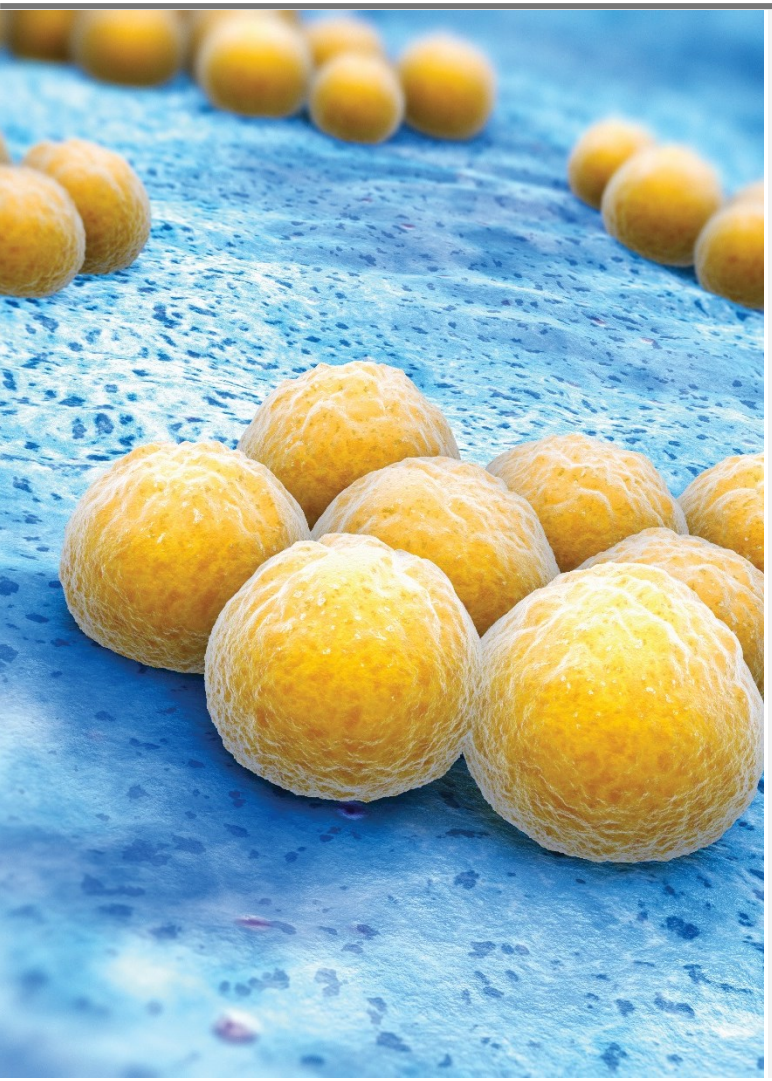
Outline

- Definition of a strain
- Why strain typing is important
- Phenotypic and genotypic methods for strain identification

Definition of a strain

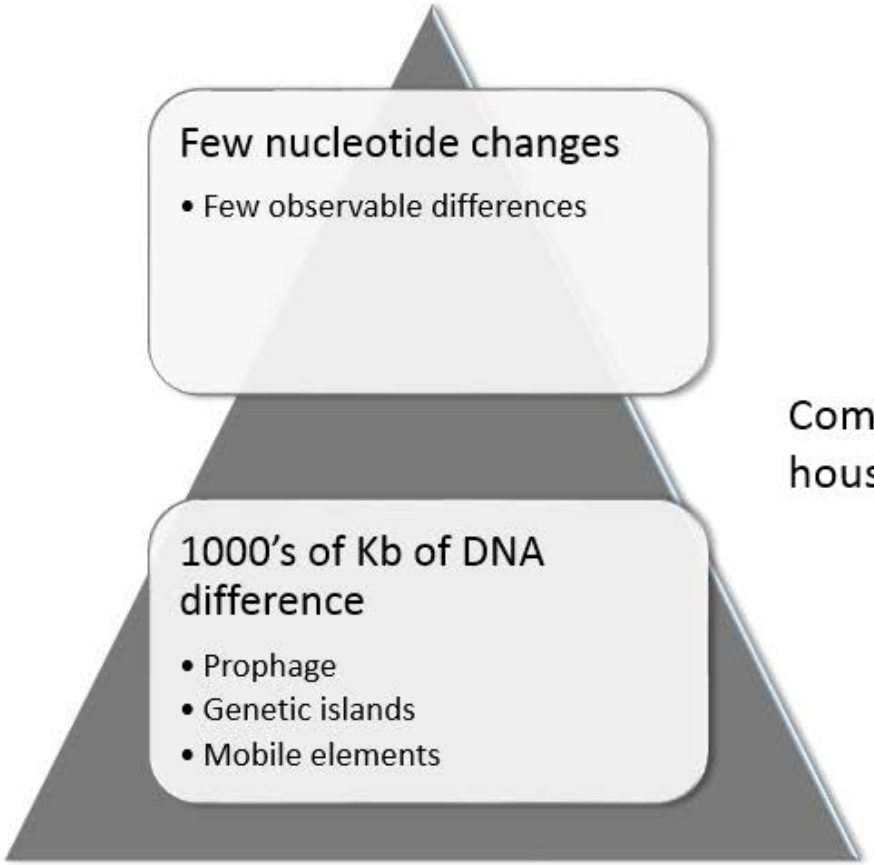


What is a “strain”?

- An individual isolate?
- A clonal population?
- A genetic variant?

A strain is a genetic variant or subtype of a microorganism below the level of species and subspecies

Strain variability



Common core of housekeeping genes

Strains of the same species can have a wide range of variation

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Why is it important to identify strains?

Identify novel isolates

- Defining type strains and variants

Clinical impact

- Identifying antimicrobial-resistant strains and virulent strains

Epidemiology

- Tracking outbreak sources and modes of transmission

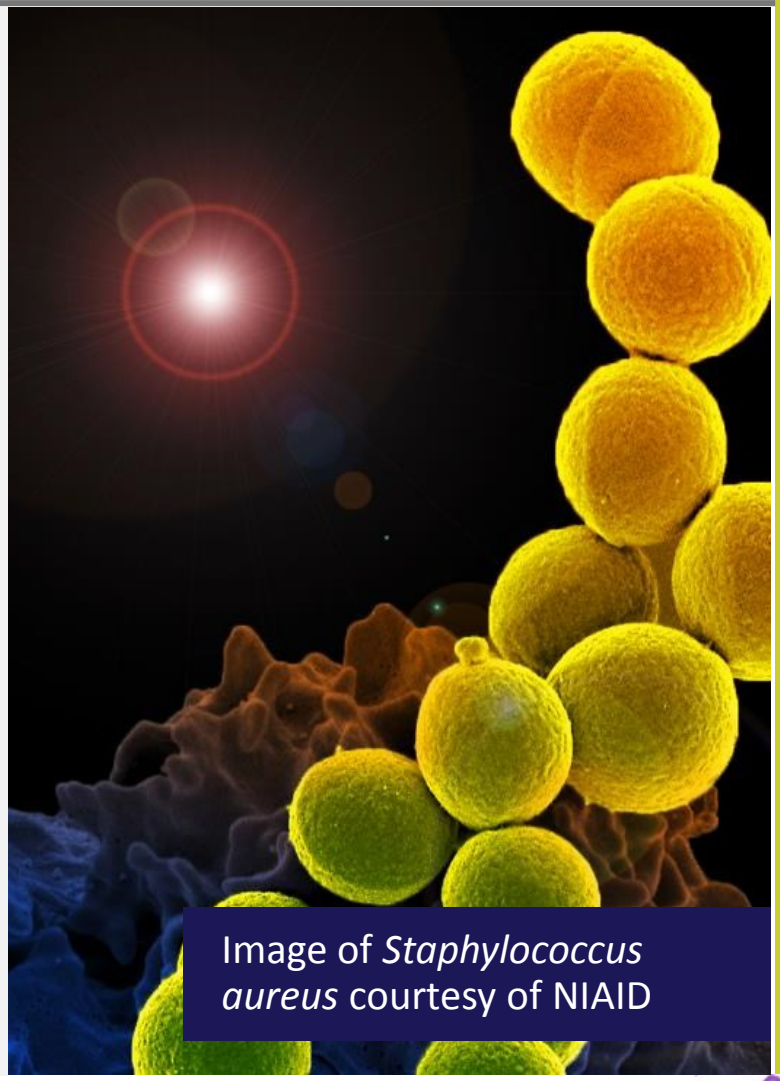

Image of *Staphylococcus aureus* courtesy of NIAID

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Identifying strains

Phenotypic Methods

- Biotyping
- Phage typing
- Serotyping

Genotypic Methods

- Electrophoretic analysis
- PCR analysis
- Sequence analysis

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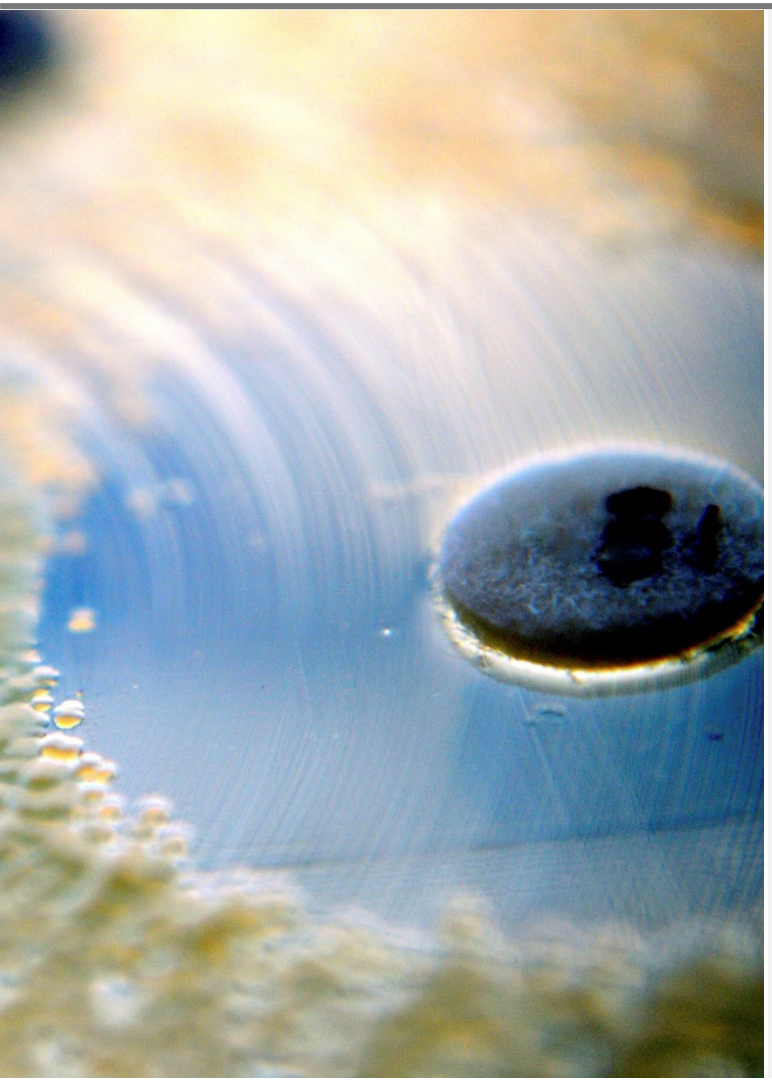
Biotyping



The use of differential tests, in addition to typical morphological and functional analyses used to determine the genus and species

- Growth in differential media
- Colony morphology
- Cell morphology
- Motility
- Antimicrobial resistance

These assays have limited discriminatory power

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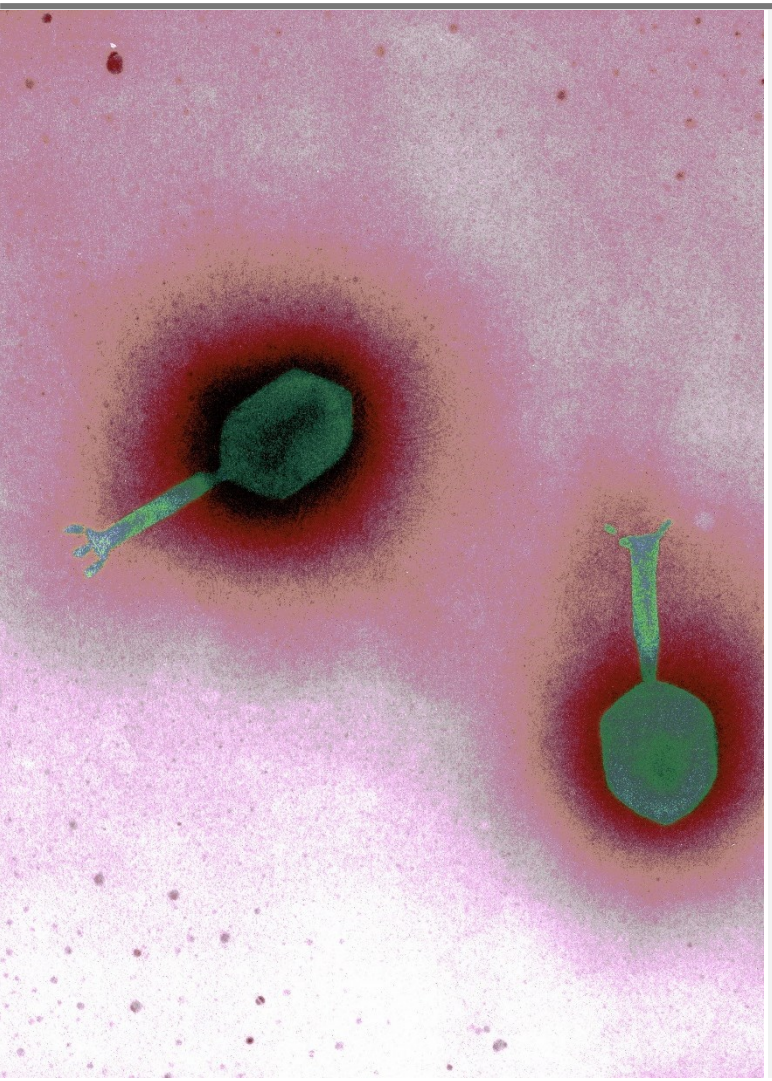
Phage typing



- Divides species into strains on the basis of susceptibility to bacteriophage lysis
- Uses common lab equipment and techniques
- Good discriminating power, but limited by panels of available phages and susceptibility to mutation

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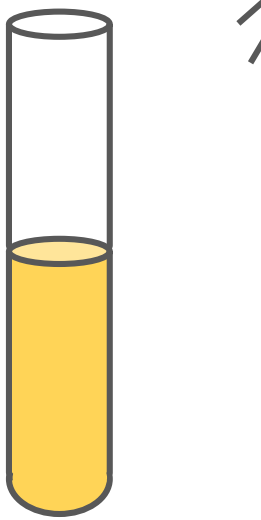
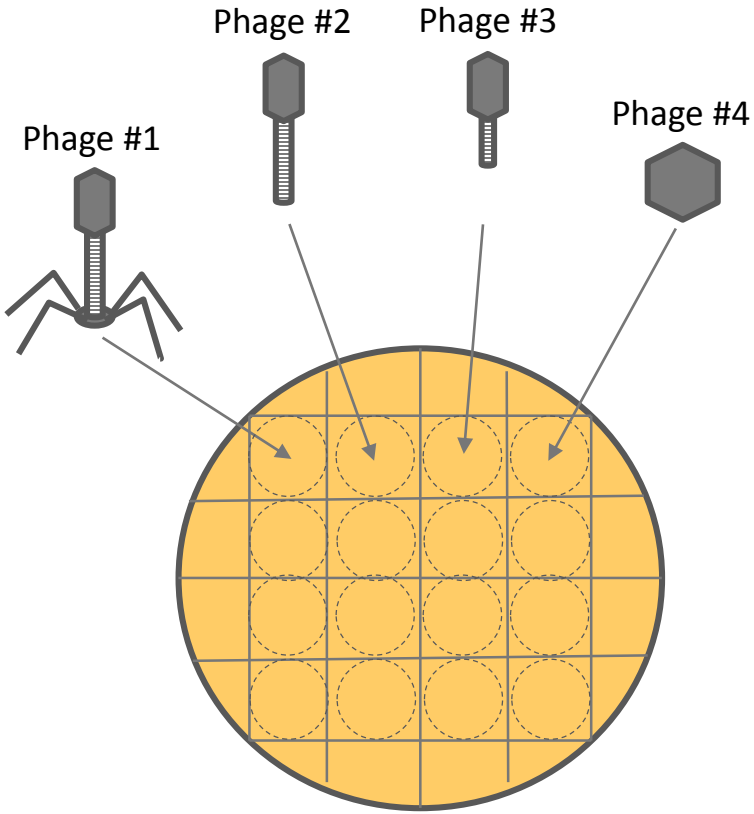
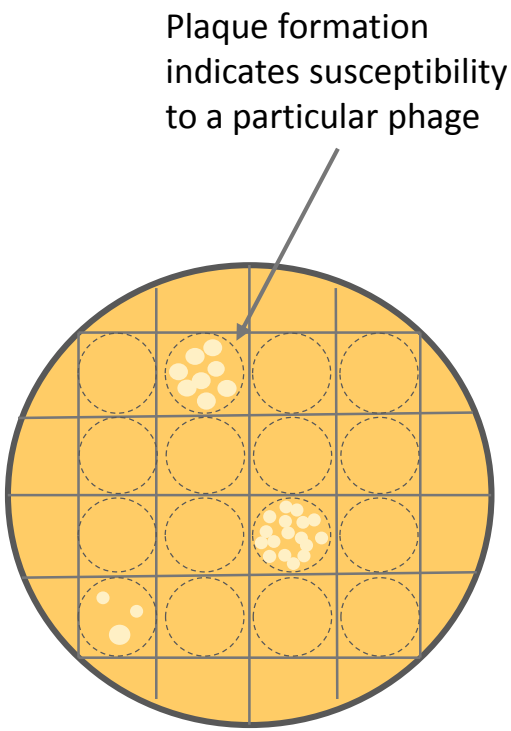
Phage typing



Prepare a lawn of the bacterial test strain



Spot with aliquots of each phage in the panel



Evaluate the pattern of phage susceptibility to identify the strain

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Serotyping

Image of *Salmonella* serotype Typhi courtesy of the CDC

- Groups strains on the basis of antigenic variation on the cell surface
- Convenient and widely used
  - Rapid and easy to perform
  - Requires the use of basic lab equipment
- Limited by the availability of antibodies against specific antigens

Serotyping

Bacterial species	Antigens
Escherichia coli	LPS oligosaccharide Flagellar subunit
Salmonella sp.	LPS oligosaccharide Flagellar subunit Capsule
Pseudomonas sp.	LPS oligosaccharide
Streptococcus pneumoniae	Capsule
Haemophilus influenza	Capsule

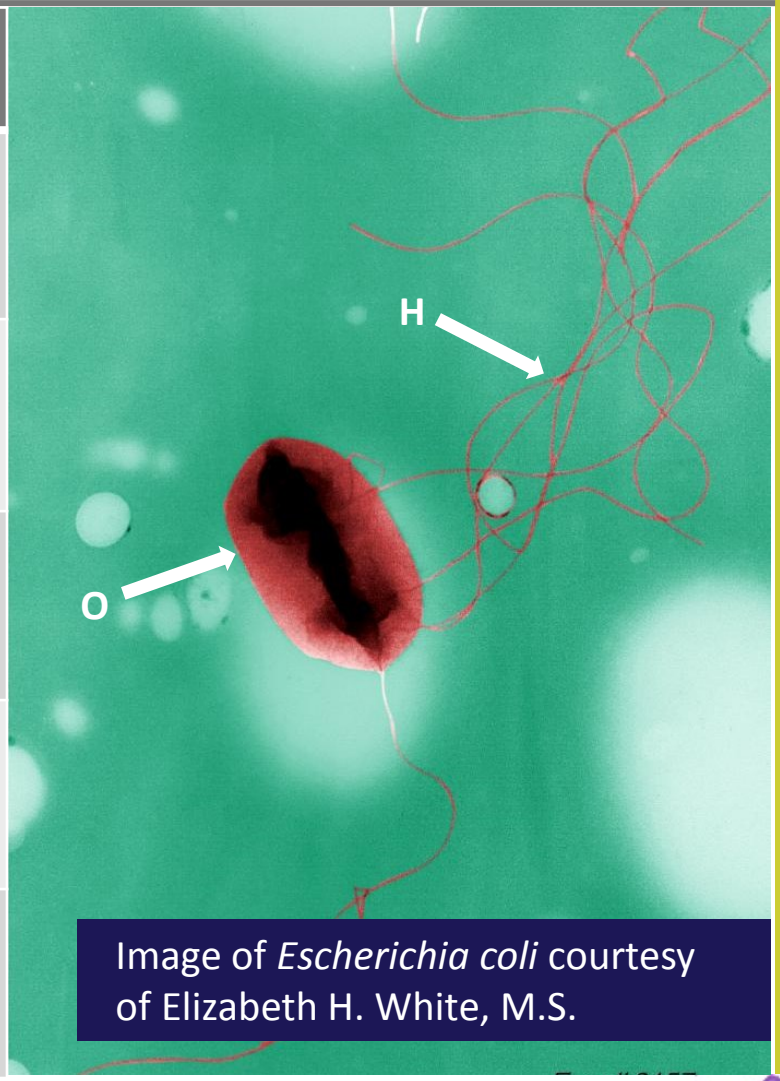
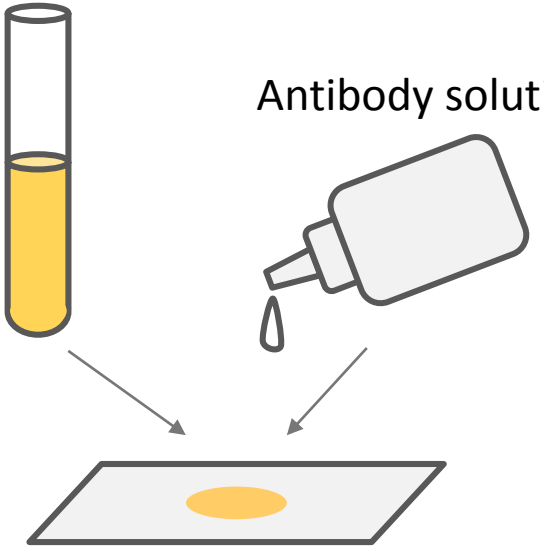


Image of *Escherichia coli* courtesy of Elizabeth H. White, M.S.

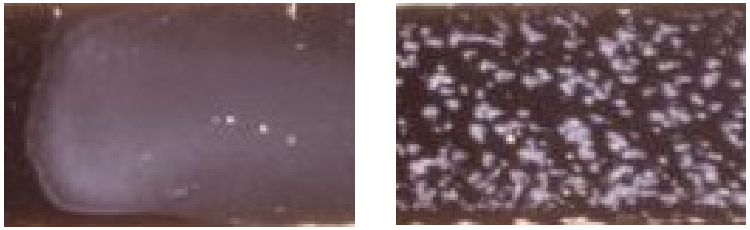
Serotyping

Culture

Antibody solution

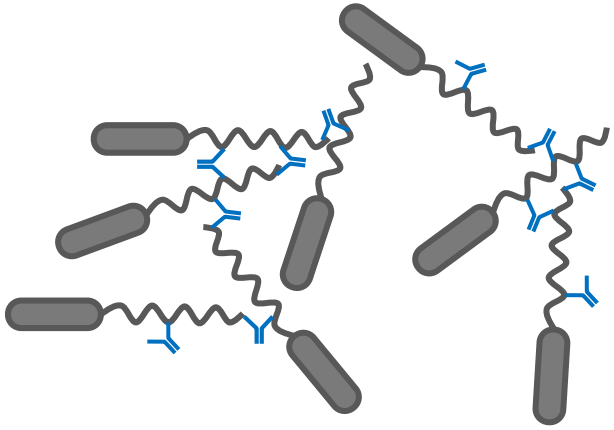


Mix

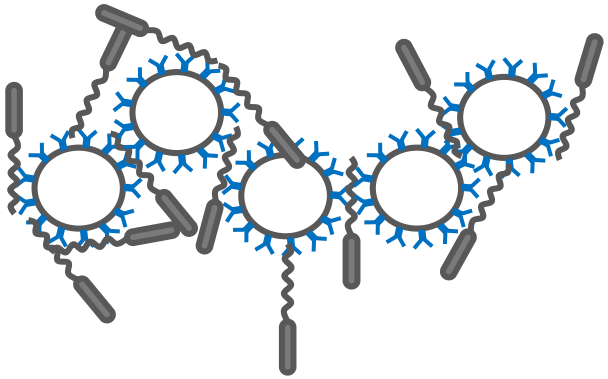


(-) (+)

Direct agglutination



Indirect agglutination
(Latex beads)



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Genotypic methods

Genome fragment size

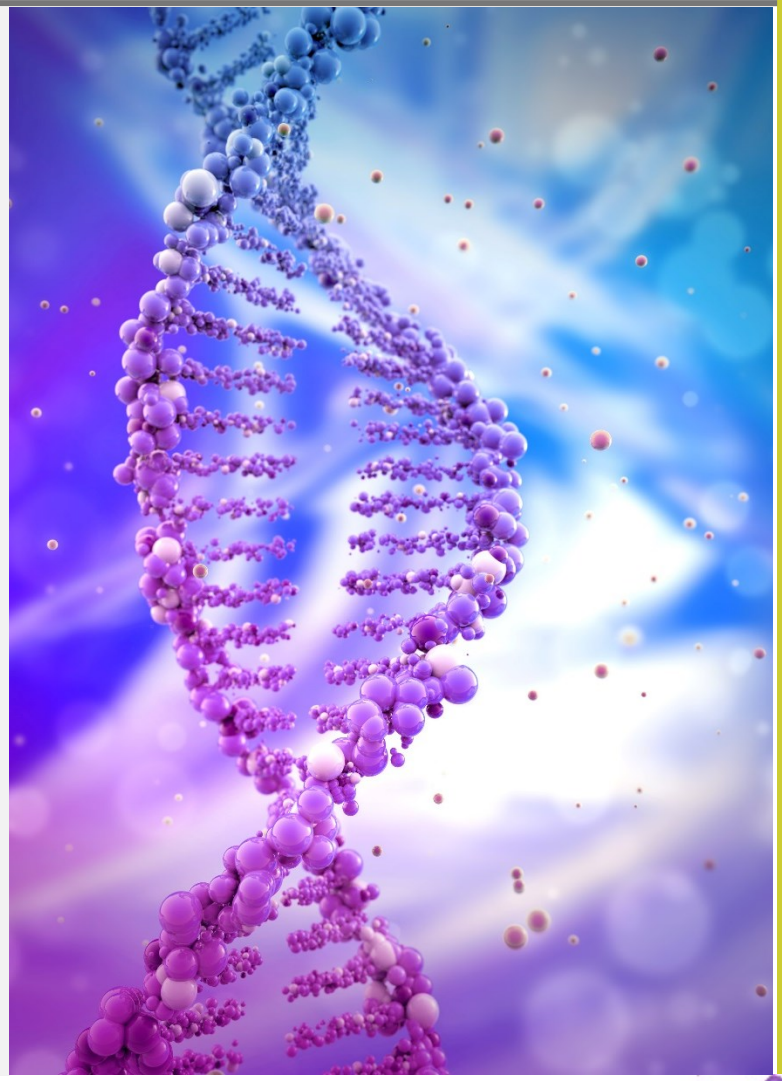
- Electrophoresis-based methods

Gene presence

- PCR-based detection of genes

Gene sequence

- Nucleotide sequence of one or more genes



Bacterial genome

Core Genome

- Set of housekeeping genes in all strains
- Evolves slowly
- Conserved positions

Accessory Genes

- Additional functions in a subset of strains
- Includes virulence genes, resistance genes, prophage, etc.
- Evolves quickly
- Frequent rearrangement, insertion/deletion

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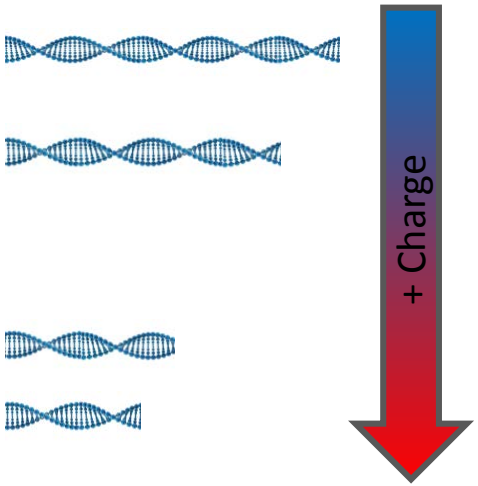
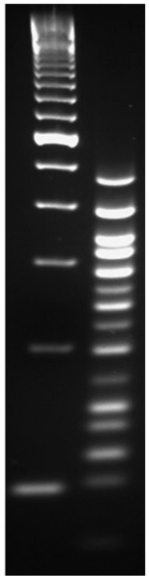
Electrophoretic analysis

Fragment genomic DNA using restriction enzymes



Separate by size using electrophoresis



Visualize fragments and compare pattern to database

Restriction fragment length polymorphism

DNA cut into small fragments

- Generates large number of fragments
- Visualized by hybridization probe

Difference in banding pattern

- Lost or gained restriction enzyme sites
- Insertions or deletions

Examples

- Ribotyping – 16S rDNA probe
- *Clostridium* toxinotyping – PCR of highly variable toxin locus

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Pulsed-field gel electrophoresis

Bacterial cells imbedded in gel plugs

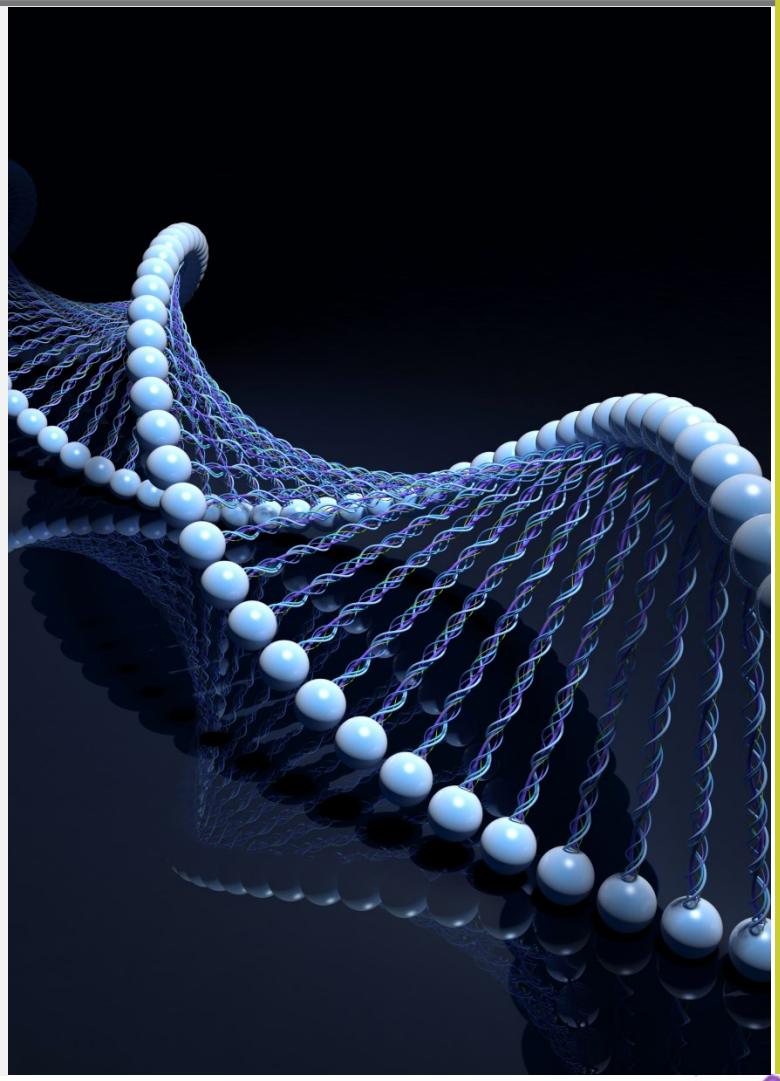
- Cells lysed and DNA digested in plugs
- Generate 10-20 large fragments

Separate DNA by using a pulsed-field gel apparatus

- Directly visualize fragments

Compare pattern to those within an established database

- Software stores and compares imaged gels



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Pulsed-field gel electrophoresis databases

PulseNet

- Established by CDC in 1996
- Searchable database of PFGE patterns
- Patterns provided by local health labs
- CDC cluster analysis looks for patterns in new uploads

PulseNet International

- Success of PulseNet led to similar efforts in other regions
- Advanced networks in Europe, Canada, etc.
- Challenges in developing world include access to PFGE equipment and data

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Multiple Locus Variable-number Tandem Repeat Analysis

Variable-number tandem repeats

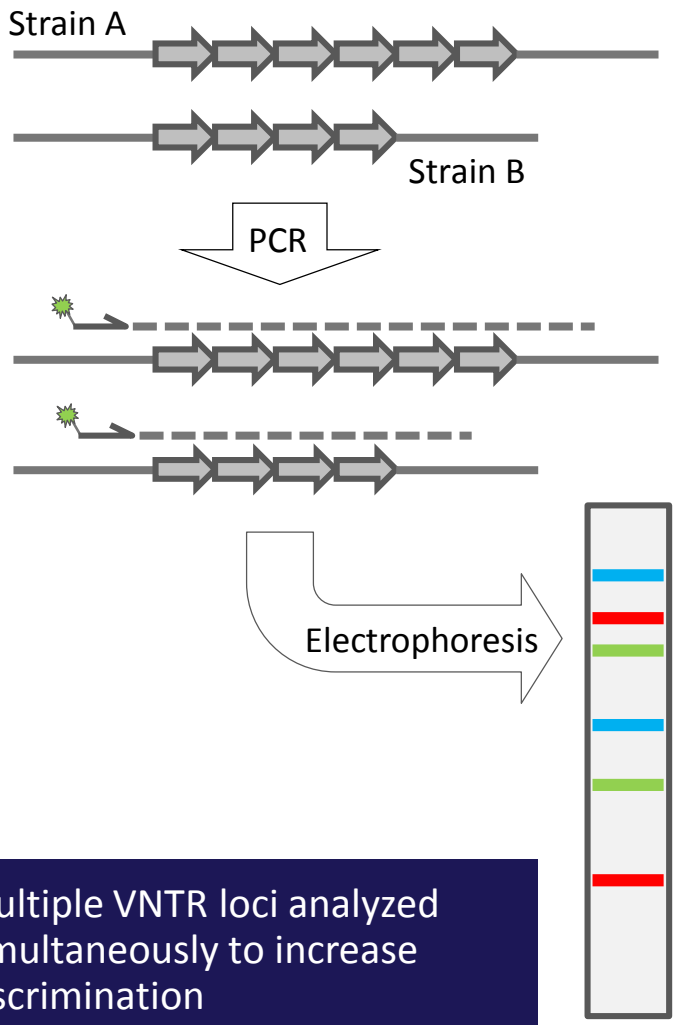
- Short repeated nucleotide sequences
- Found in most organisms
- The number of repeats varies between strains

MLVA

- Amplify tandem repeat loci by PCR and separate by electrophoresis
- Visualize using fluorescently labeled primers

MLVA resources

- PulseNet, MLVABank
- Strain databases and protocols for different MLVA equipment
- PulseNet Databases include *Salmonella* and *E. coli* O157:H7
- MLVABank has databases for ~20 species



Multiple VNTR loci analyzed simultaneously to increase discrimination

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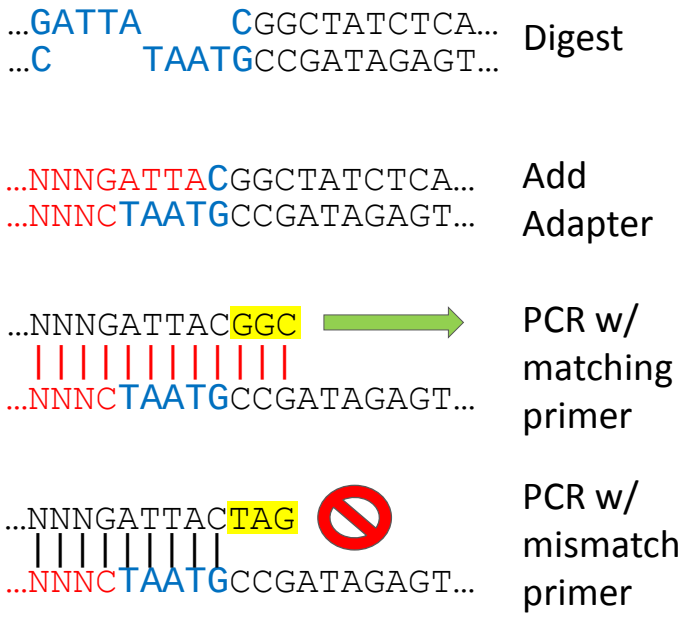
Amplified Fragment Length Polymorphism



Base pairs at end of primer match to subset of fragments

3 base extension will match ~1/64 fragments

Process DNA with RFLP

- Frequent restriction sites
- Many smaller fragments (~50 to ~1,000 bp)

Visualize subset of fragments

- Non-specific adapter annealed to the restriction site
- PCR primers bind adapter and restriction sites
- Separate fragments by electrophoresis and visualize

No prior sequence knowledge required

- Environmental samples
- Eukaryotes
- Commercial kits

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Comparative genomic fingerprinting

Targets the presence of accessory genes

- Target selection enabled by the availability of genome sequences

Multiplex PCR and electrophoresis to detect ~40 genes

Isolates groups into subtypes based on patterns of genes present

- Assays available for *Campylobacter, Arcobacter*, and *Escherichia*

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Gene sequence

DNA template and primer

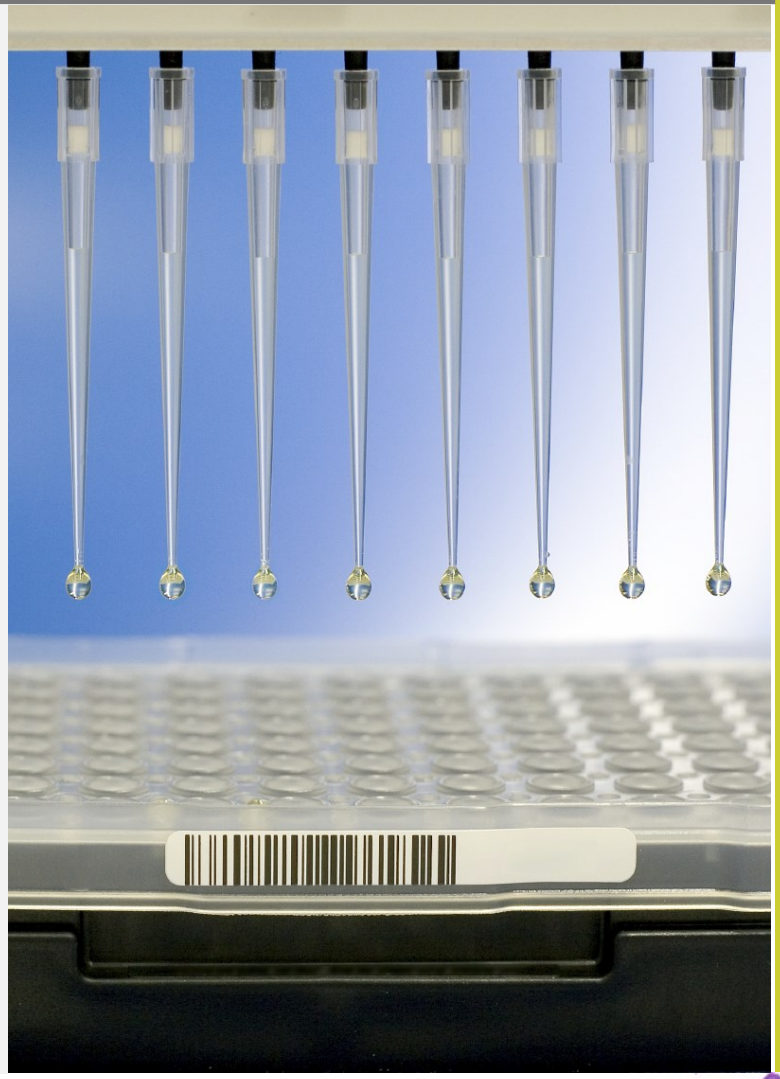
- Plasmid DNA
- Amplified PCR product
- Specific oligonucleotide primer

Amplification

- Incorporates nucleotides labeled with fluorescent dyes
- Sequence length up to ~700 bp

Analysis

- Fluorescent dyes detected to determine nucleotide sequence
- Sequence results compared to sequence databases – NCBI, etc.



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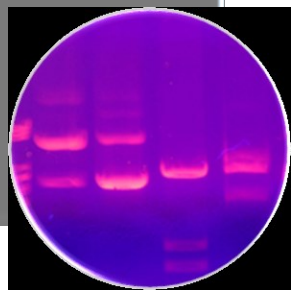
Gene sequence

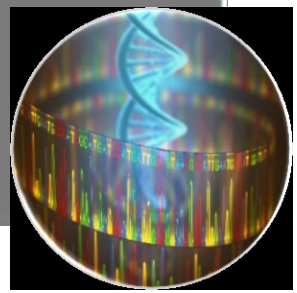
- Detects sequence changes that produce:
  - Altered restriction sites
  - Changes in size

Electrophoresis



- Detects all sequence changes within the area examined

Sequencing



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Gene sequence

Methicillin-resistant *Staphylococcus aureus*

- *spa*
- SCC*mec*

Clostridium difficile

- *slpA* (molecular serotyping)

Rickettsia

- *ompA*

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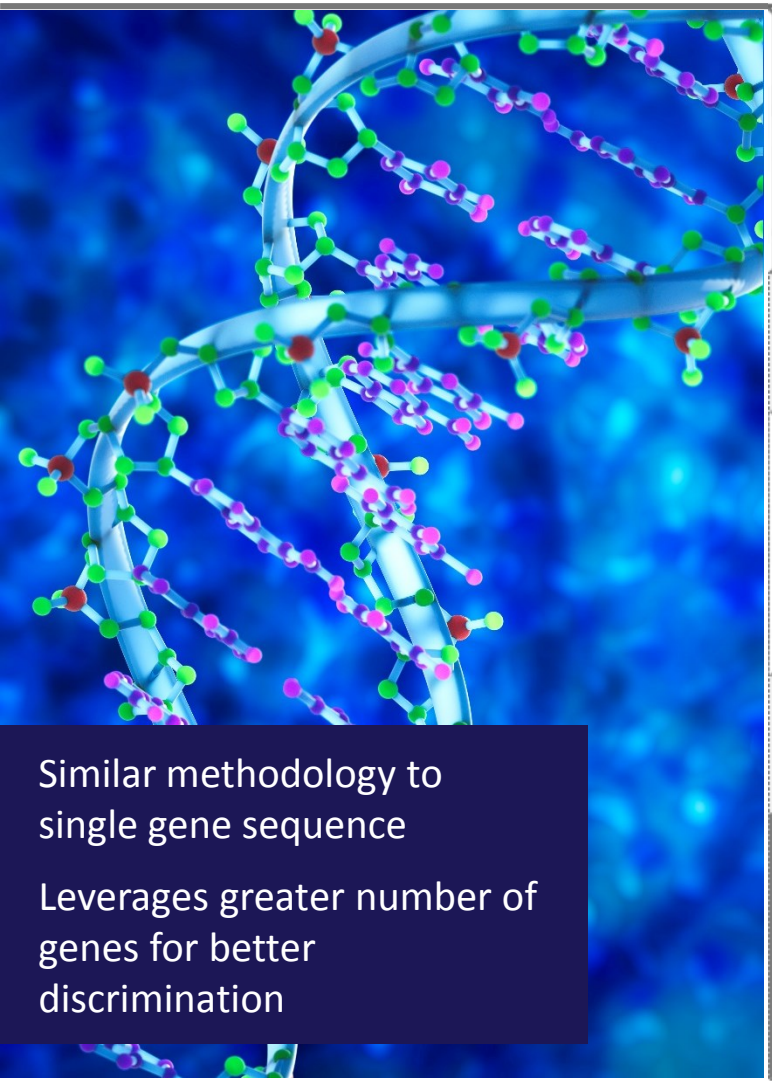
Multilocus sequence typing



Similar methodology to single gene sequence

Leverages greater number of genes for better discrimination

PCR amplify and sequence the defined regions of housekeeping genes – usually 7

Assign the allele number to each unique DNA sequence of each gene

Compare to database to assign sequence type number to each unique combination of alleles

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Multilocus sequence typing

MLST databases

- Establish primer sets to define sequenced regions of each species
- Collect allele sequences and combinations from researchers
- Define sequence types for allele combinations
- Gather databases from multiple species into general MLST

MLST information

- Easy to store sequence text versus gel images
- Easy and fast to search text files

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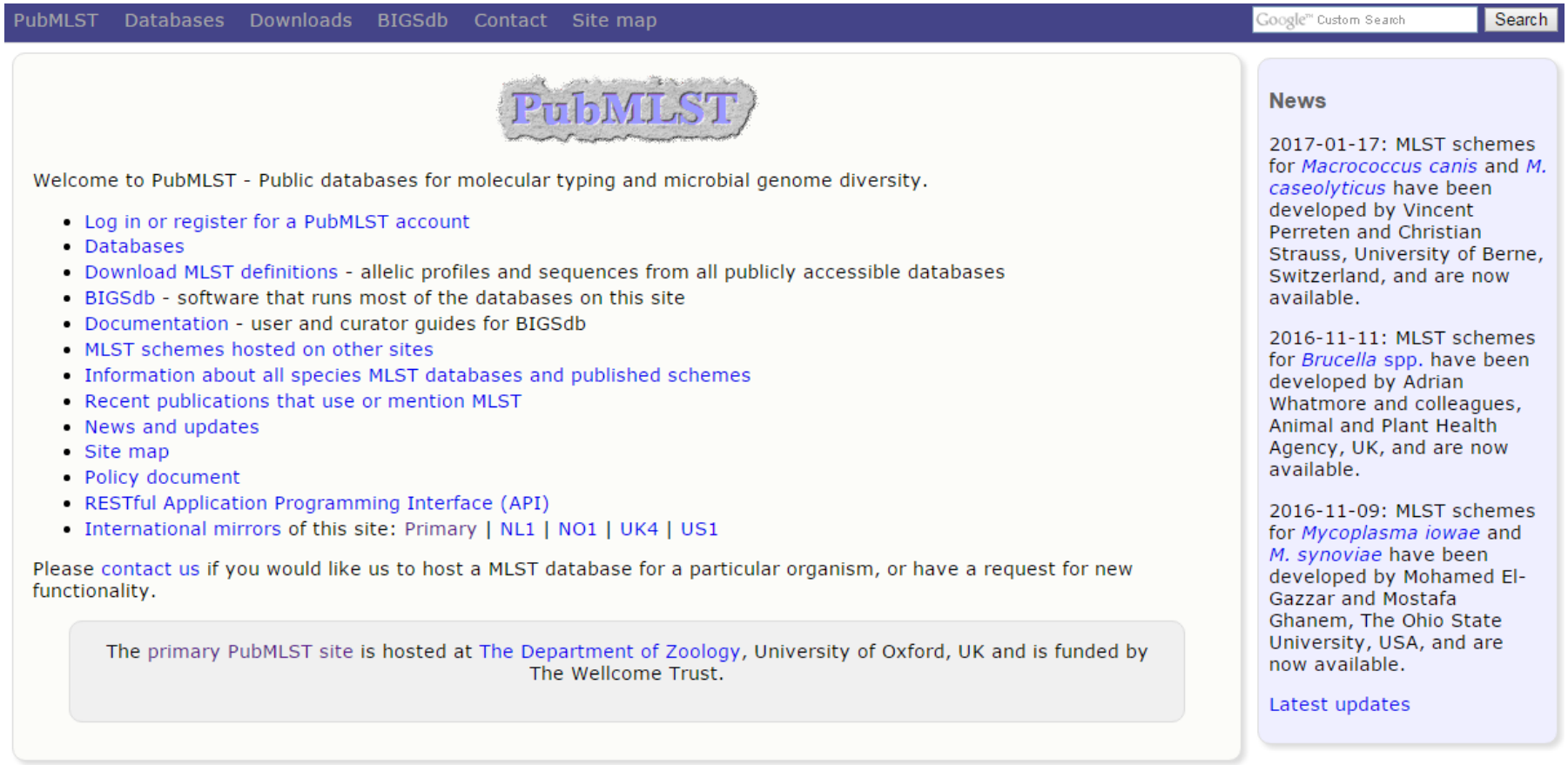
Multilocus sequence typing database



http://pubmlst.org/

University of Oxford, Wellcome Trust

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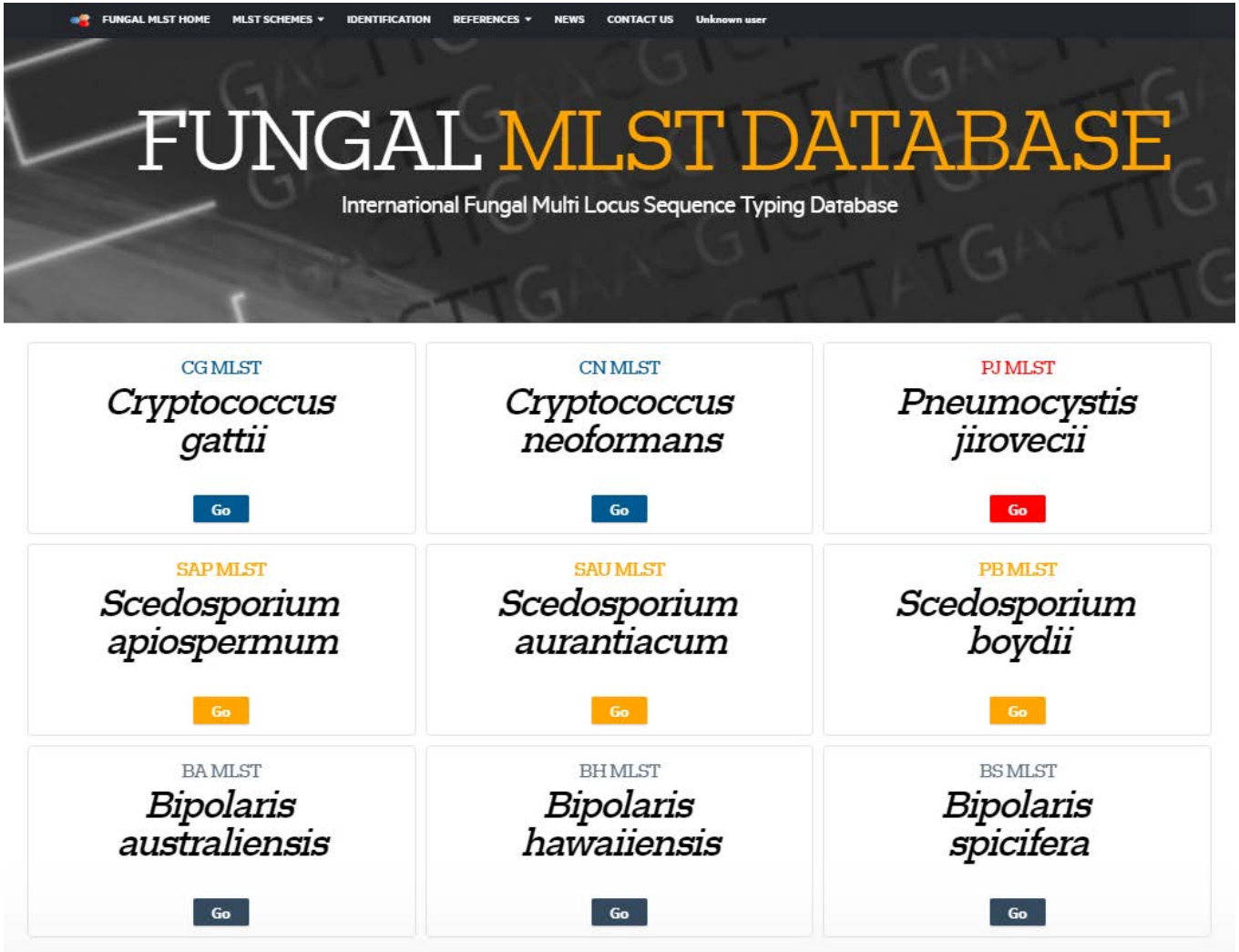
Multilocus sequence typing database



http://mlst.mycologylab.org/

Multilocus sequence typing

Example species available in PubMLST databases

Bacteria			Eukaryotes
Escherichia coli #1	*Acinetobacter baumannii*	*Bacillus cereus*	*Candida albicans*
Enterobacter cloacae	*Borrelia* spp.	*Staphylococcus aureus*	*Candida tropicalis*
Salmonella enterica	*Burkholderia pseudomallei*	*Streptococcus pneumoniae*	*Aspergillus fumigatus*
Klebsiella pneumoniae	*Campylobacter jejuni*	*Arcobacter* spp.	*Penicillium marneffei*
Yersinia pseudotuberculosis	*Helicobacter pylori*	*Wolbachia* spp.	*Kudoa septempunctata*
Vibrio parahaemolyticus	*Neisseria* spp.	*Xylella fastidiosa*	*Trichomonas vaginalis*
92 MLST schemes			**9 MLST schemes**

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Multilocus sequence typing

PubMLST Database home Contents

Log in

Download allele sequences

Select loci by scheme | Alphabetical list | All loci by scheme

MLST

Locus	Download	Type	Alleles	Length (setting)	Min length	Max length	Full name/product	Curator(s)	Last updated
arcC		DNA	436	Fixed: 456 bp	435	457		K. Jolley	2017-01-23
aroE		DNA	588	Fixed: 456 bp	453	456		K. Jolley	2017-01-23
glpF		DNA	523	Fixed: 465 bp	450	466		K. Jolley	2017-01-23
gmk		DNA	297	Fixed: 417 bp	417	420		K. Jolley	2017-01-30
pta		DNA	468	Fixed: 474 bp	474	474		K. Jolley	2017-01-31
tpi		DNA	429	Fixed: 402 bp	402	402		K. Jolley	2017-01-30
yqiL		DNA	511	Fixed: 516 bp	516	516		K. Jolley	2017-01-23

Staphylococcus aureus

31,885 isolates
3,775 sequence types

ST	arcC	aroE	glpF	gmk	pta	tpi	yqiL
1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	26
3	1	1	1	9	1	1	12
4	10	10	8	6	10	3	2
5	1	4	1	4	12	1	10
6	12	4	1	4	12	1	3
7	5	4	1	4	4	6	3
8	3	3	1	1	4	4	3
9	3	3	1	1	1	1	10
10	8	7	6	2	9	9	7
11	1	24	1	4	12	1	10
12	1	3	1	8	11	5	11
13	1	3	1	10	11	5	11
14	1	13	1	1	12	11	13

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http://pubmlst.org/

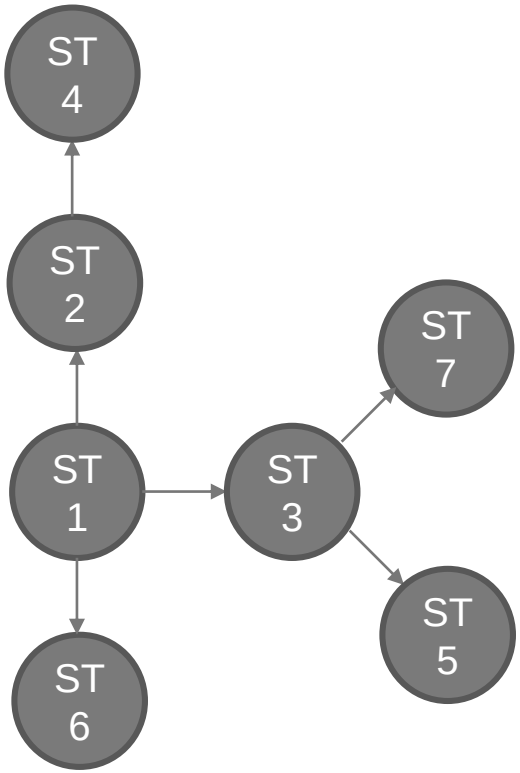
University of Oxford, Wellcome Trust

Multilocus sequence typing

Sequence types are grouped together on the basis of similarity

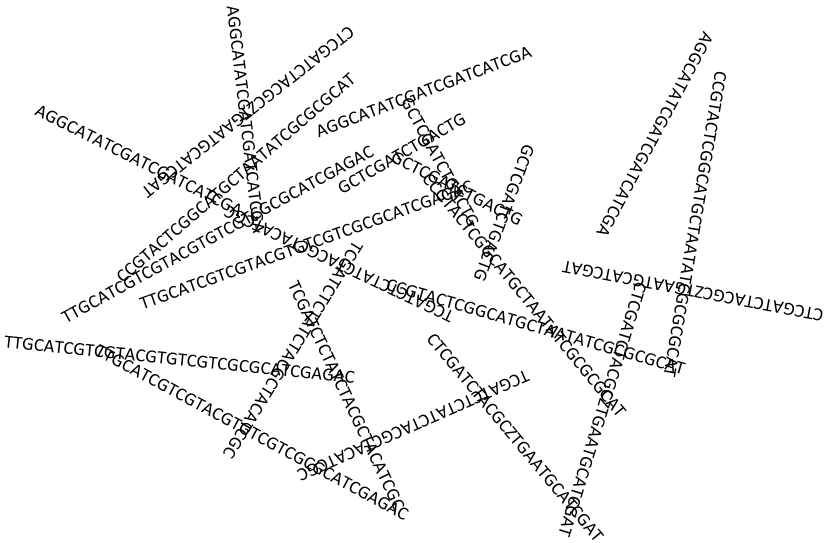
- Identical at number of alleles

Can be used to track changes to a population



Each arrow represents a change at one locus relative to the original sequence type

Whole genome sequence



- Fragments are aligned and assembled into larger contigs
- Pyrosequencing generates a very large number of small (~30-50 bp) fragments
  - Automated process
- Contigs are assembled to produce finished chromosomes
  - Manual process

CCGTACTCGGCATGCTAATATCTCGATCTA
CTCGATCTACGCZTGAATGCATCGAT
TCGATTATCGATCGATCATCGA

…CCGTACTCGGCATGCTAATATCTCGATCTACGCZTGAATGCATCGATTATCGATCGATCATCGA…

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Whole genome sequence

Multi-locus analysis

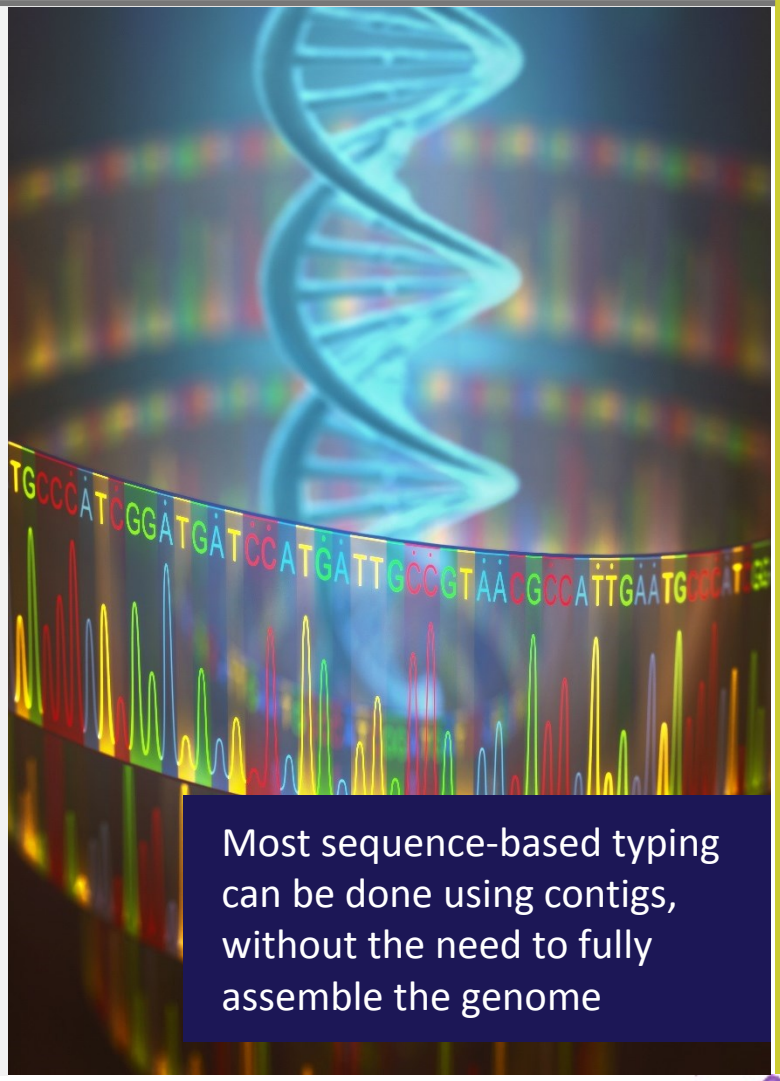
- MLST
- Pairwise SNP comparison
- Phylogenetic analysis

Single locus analysis

- Single gene typing (*e.g.* MRSA *spa*)
- Molecular typing
  - Identify serotype by specific alleles

Molecular detection of phenotypic typing markers

- Virulence genes, antimicrobial resistance genes, biochemical pathways, phage receptors



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Whole genome sequence

Limitations to whole genome sequencing

- Cost
- Bioinformatics expertise

Currently used by national labs

- Centers for Disease Control and Prevention
- United States Department of Agriculture

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Summary

- Strain typing identifies characteristics that differ between isolates of the same species
- Phenotypic typing methods, such as serotyping, offer speed and ease of use
- Genetic typing methods assess strain differences at the DNA level
  - Electrophoretic techniques look for changes in DNA fragment patterns
  - Sequencing techniques directly analyze the DNA sequence of one or more genes
- Genetic typing offers increasing levels of discrimination but requires more specialized equipment and training

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Disclaimers

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