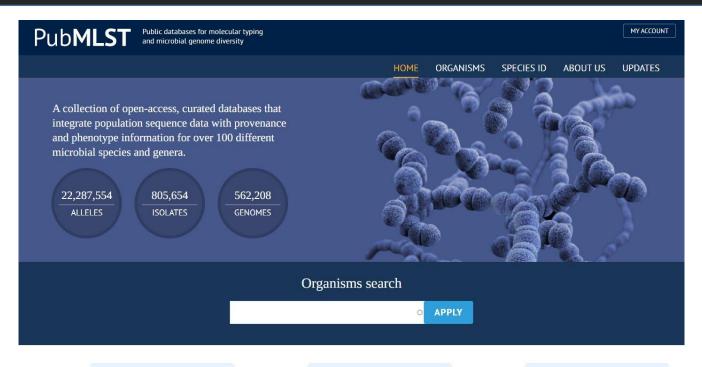
# **PubMLST**

Ho Phu Quy

### What is PubMLST?



PubMLST (Public Multi-Locus Sequence Typing) is a comprehensive online database and bioinformatics platform designed for the storage, analysis, and dissemination of molecular typing data for bacterial species



#### **Organisms**

Choose your organism from a list of over 100 species and genera-specific databases.

Access molecular typing and isolate records.



#### Species ID

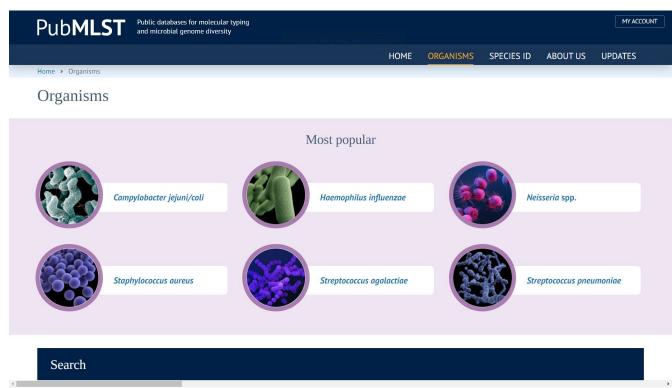
Use ribosomal MLST to accurately identify bacterial species from a genome assembly.



#### Submit data

We welcome submissions to the databases we host. Submissions may consist of new allele sequences, MLST profiles, or isolate records. Isolates may be accompanied by a genome assembly.

### What is PubMLST?



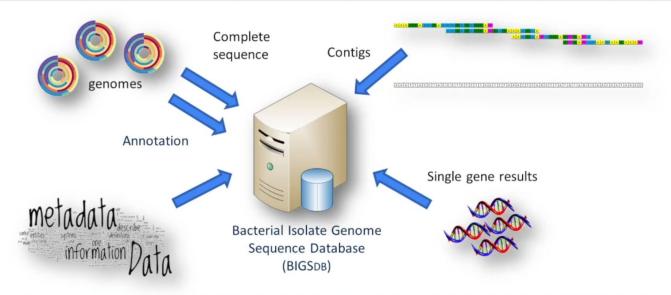
ubMLST (Public Nulti-Locus Sequence yping) have 139 rganisms.

ubMLST hosts databases or a wide range of bacterial pecies, providing tools for nolecular typing and pidemiological analysis. ach organism has a edicated database ontaining allelic profiles, equence types (STs), and netadata.

## What is PubMLST?

# Population genomics: the BIGSdb platform





Jolley & Maiden 2010. BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* **11:**595

Home > Organisms > Staphylococcus aureus

### S. aureus clonal complex designation

We have identified the common, largely non-overlapping clonal complexes within the database associated with ST. Clonal complexes here are defined as those STs that match the central genotype (ST) at four or more loci unless they more closely match another central genotype.

STs are assigned to clonal complexes automatically by a script that runs against the database each evening.

### Currently defined complexes

Central ST	Complex name
1	CC1
5	CC5
8	CC8
15	CC15
22	CC22
30	CC30
45	CC45
97	CC97
93	CC93
121	CC121

This is not an exhaustive list of complexes, but those that are seen most frequently in the database and can usually be

https://pubmlst.org/organisms/staphylococcus-aureus/clonal-complexes

Home > Organisms > Staphylococcus aureus

### Staphylococcus aureus MLST primers

#### Genes

The S. aureus MLST scheme uses internal fragments of the following seven house-keeping genes:

- arcC (Carbamate kinase)
- aroE (Shikimate dehydrogenase)
- glpF (Glycerol kinase)
- gmk (Guanylate kinase)
- pta (Phosphate acetyltransferase)
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- · yqi (Acetyle coenzyme A acetyltransferase)

### Staphylococcus aureus DNA extraction protocol

- 1. Resuspend 1/8 of a plate of overnight confluent growth from a blood agar plate in 400  $\mu$ l lysis solution (see below)
- 2. Incubate at 37 °C 30 min.
- 3. Heat to 95 °C for 10 min.
- 4. Place on ice and add 1 ml phenol/chloroform isoamyl alcohol (25:24:1) and mix thoroughly by inversion.
- 5. Centrifuge 10,000 g for 20 min.
- 6. Recover aqueous layer to fresh tube and precipitate DNA with the addition of 1 ml absolute ethanol.
- 7. Place on ice for 15 min then pellet DNA by centrifugation at 10,000g for 20 min.
- 8. Resuspend DNA in 50 µl water.

### Lysis solution

- 0.5 ml 5000 units/ml lysozyme
- 0.5 ml 500 units/ml lysostaphin
- 0.2 ml 0.5M EDTA
- 0.1 ml 1M Tris
- · 8.7 ml de-ionised water

### PCR conditions

PCR amplification is carried out on chromosomal DNA using an extension time of 30 s, and an annealing temperature of 55 °C, with Qiagen 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.

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https://pubmlst.org/organisms/staphylococcus-aureus/primers

Home > Organisms > Staphylococcus aureus

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glp up - 5' CTA GGA ACT GCA ATC TTA ATC C -3'
glp dn - 5' TGG TAA AAT CGC ATG TCC AAT TC -3'
gmk up - 5' ATC GTT TTA TCG GGA CCA TC -3'
gmk dn - 5' TCA TTA ACT ACA ACG TAA TCG TA -3'
pta up - 5' GTT AAA ATC GTA TTA CCT GAA GG -3'
pta dn - 5' GAC CCT TTT GTT GAA AAG CTT AA -3'
tpi up - 5' TCG TTC ATT CTG AAC GTC GTG AA -3'
tpi dn - 5' TTT GCA CCT TCT AAC AAT TGT AC -3'
ygi up- 5' CAG CAT ACA GGA CAC CTA TTG GC -3'
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glp dn - 5' TGG TAA AAT CGC ATG TCC AAT TC -3'
gmk up - 5' ATC GTT TTA TCG GGA CCA TC -3'
gmk dn - 5' TCA TTA ACT ACA ACG TAA TCG TA -3'
pta up - 5' GTT AAA ATC GTA TTA CCT GAA GG -3'
pta dn - 5' GAC CCT TTT GTT GAA AAG CTT AA -3'
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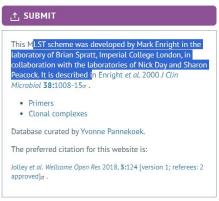
https://pubmlst.org/organisms/staphylococcus

-aureus/primers



### Staphylococcus aureus





#### Typing

The typing database contains nomenclature - allele definitions that provide an identifier for every unique allele sequence, and MLST profiles that index each unique combination of alleles with a sequence type (ST).

Allele sequences: 954,696

Last updated: 2024-06-13

#### Isolate collection

The isolate database consists of isolate records containing provenance and phenotype information linked to molecular typing information. These records may also include genome assemblies.

Isolates: 42,787

Last updated: 2024-06-19

#### Genome collection

A subset of records within the isolate database may contain genomes assemblies. You can access these from the isolate database by filtering on sequence bin size in a query.

Genomes: 30,785

Last updated: 2024-06-12

https://pubmlst.org/organisms/staphylococcus

-aureus

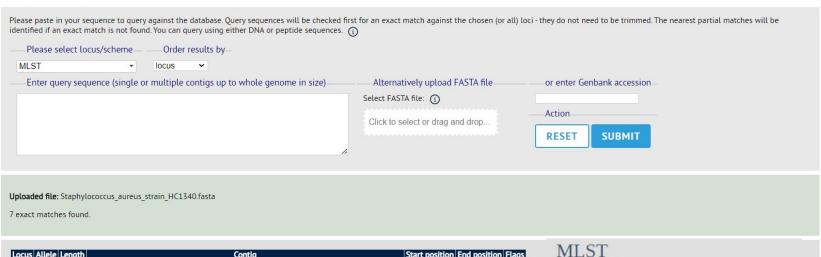
Home > Organisms > Staphylococcus aureus > Staphylococcus aureus typing > Sequence query







### Sequence query



Locus	Allele	Length	Contig	Start position	End position Flag
arcC	2	456	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome,_complete_genome	2948666	2949121
aroE	3	456	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome,_complete_genome	1832909	1833364
glpF	1	465	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome,_complete_genome	1448319	1448783
gmk	1	417	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome,_complete_genome	1342835	1343251
pta	4	474	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome, complete_genome	731881	732354
tpi	4	402	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome,_complete_genome	933071	933472
yqiL	3	516	NZ_CP012011.1_Staphylococcus_aureus_strain_HC1340_chromosome,_complete_genome	462415	462930

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.



Matching profile

ST: 239

clonal complex: CC8



### Sequence attribute search



You can also search using the locus-specific query page. Use this page for access to the sequence analysis or export plugins. Please enter your search criteria below (or leave blank and submit to return all records). Matching sequences will be returned and you will then be able to update their display and query settings. Search criteria Display **~** = ✓ arcC + (i) Order by: locus ✓ ascending ✓ locus Display: 25 v records per page (i) Filter query by Action SEARCH

1,044 records returned (1 - 25 displayed). Click the hyperlinks for detailed information.





1 2 3 4 5 6 (>) (>>)



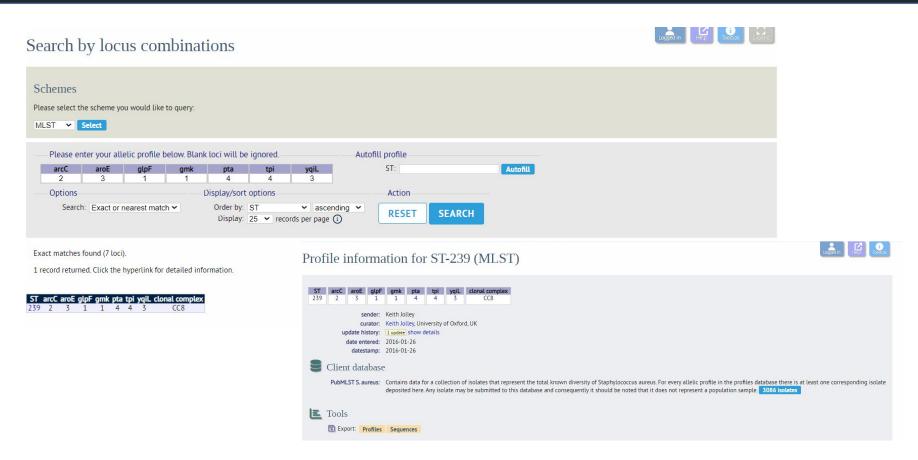


locus a	ıllele id	sequence	sequence length	type allele flags
arcC	1	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	2	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	3	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	4	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	5	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	6	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	7	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	8	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	9	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	10	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	11	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	12	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	13	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	14	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	15	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	16	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	17	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	18	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	19	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	20	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	21	TTATTAATCCAACAA GAAGGTGTTGAAGCG	453	
arcC	22	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	23	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	24	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	
arcC	25	TTATTAATCCAACAA GAAGGTGTTGAAGCG	456	

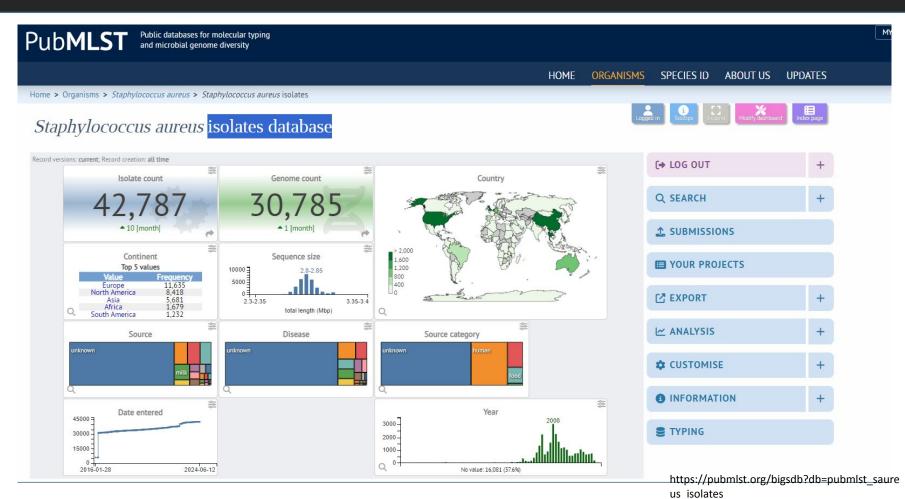








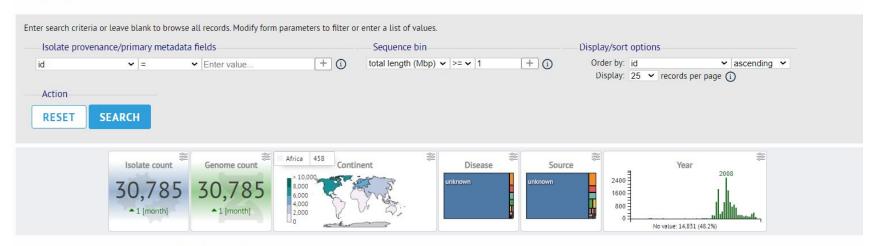
# Isolates collection



# Genome collection

### Search or browse database

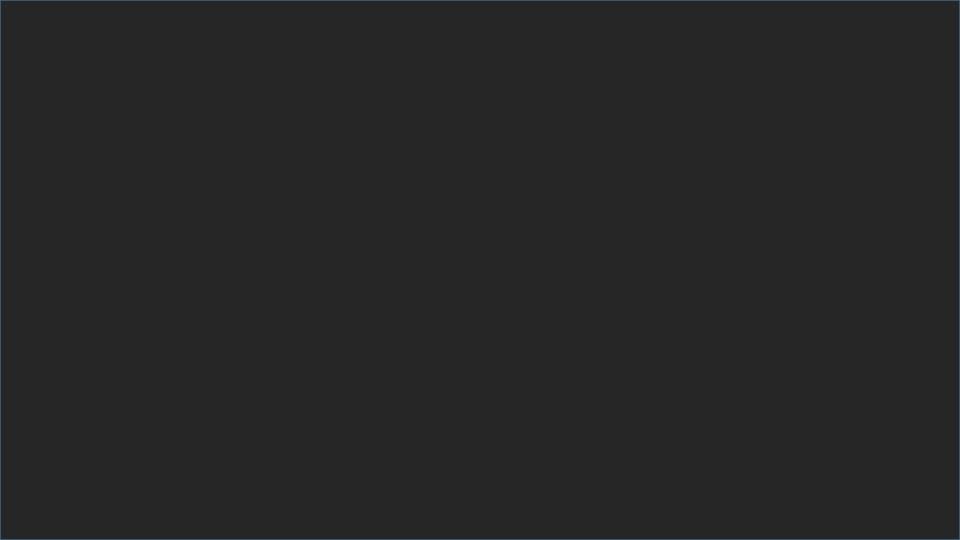




30,785 records returned (1 - 25 displayed). Click the hyperlinks for detailed information.

	Isolate fields 🔑					MLST										
id	isolate	aliases	country	year	disease	source category	source	arcC	aroE	glpF	gmk	pta	tpi	yqiL	ST	clonal complex
3532	Saitama9		Japan	2012	other	human	pus	3	124	1	1	1	410	40	1558	CC97
3533	Saitama11		Japan	2013	other	human	pus	3	549	1	1	1	1	40	1566	CC1
3625	ERR1197981		Unknown		unknown	unknown	unknown	2	2	2	2	6	3	2	30	CC30
3882	ERR410047		Unknown		unknown	unknown	unknown	2	2	2	2	2	2	2	39	CC30
5329	ERR410050		Unknown		unknown	unknown	unknown	3	3	1	1	4	4	3	8	CC8
5959	ERR410059		Unknown		unknown	unknown	unknown	8	2	2	2	6	3	2	34	CC30
5960	ERR410074		Unknown		unknown	unknown	unknown	8	2	2	2	6	3	2	34	CC30
5965	ERR410082		Unknown		unknown	unknown	unknown	1	1	1	1	1	1	1	1	CC1
5966	ERR410106		Unknown		unknown	unknown	unknown	7	6	1	5	8	8	6	22	CC22
6087	ERR211639		Unknown		unknown	unknown	unknown	6	57	45	2	7	58	52	130	
6088	ERR163446		Unknown		unknown	unknown	unknown	14	16	11	2	13	336	14	3121	
6089	SRR445025	199/08	Slovenia	2008	unknown	unknown	unknown	3	35	19	2	20	26	39	398	

https://pubmlst.org/bigsdb?db=pubmlst\_saure us\_isolates&page=query&genomes=1



# Activity

### Run demo workflow for coregenome-MLST analysis

Data using: 50 genome example downloaded from ncbi. (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=1280)

Tools using:

<u>chewbbaca(https://chewbbaca.readthedocs.io/en/latest/user/getting\_started/overview.html</u>) and PHYLOViZ Online(https://online.phyloviz.net/index)

Exercise: using this workflow to run dataset.

Link: https://zenodo.org/records/10694715/files/chewBBACA\_tutorial.zip