20 years of Whole Genome Sequencing (WGS) of bacteria

- Robust data: one method for all bacterial species
- Data storage for later analysis
- Monitoring of epidemic cases in hospital
- Monitoring emergency prevalent and emerging clones
- Identification of all genes of interest
- International comparison of prevalent and emerging clones

20 years of bacterial genome sequencing





Nature Reviews | Microbiology

Loman, N., Pallen, M. Twenty years of bacterial genome sequencing. *Nat Rev Microbiol* **13**, 787–794 (2015). https://doi.org/10.1038/nrmicro3565

Whole-genome sequencing

2nd generation





Illumina sequencing

G

20 years of bacterial genome sequencing





Nature Reviews | Microbiology

Loman, N., Pallen, M. Twenty years of bacterial genome sequencing. *Nat Rev Microbiol* **13**, 787–794 (2015). https://doi.org/10.1038/nrmicro3565

ION TORRENT Technology



Ion Torrent[™] technology directly translates chemically encoded information (A, C, G, T) into digital information (0, 1) on a semiconductor chip. This approach marries simple chemistry to proprietary semiconductor technology



https://www.thermofisher.com/it/en/home/life-science/sequencing/next-generationsequencing/ion-torrent-next-generation-sequencing-technology.html



Nanopore sequencing







DNA can be sequenced by threading it through a microscopic pore in a membrane. Bases are identified by the way they affect ions flowing through the pore from one side of the membrane to the other.



Nanopore sequencing



Nanopore sequencing: long reads but mistakes!!!





The average coverage for a whole genome can be calculated from the length of the original genome (G), the number of reads (N), and the average read length (L) as

$N \times L/G$.

For example, a hypothetical genome with 2,000 base pairs reconstructed from 8 reads with an average length of 500 nucleotides will have

8 x 500 : 2000 = 2× redundancy.

This parameter also enables one to estimate other quantities, such as the percentage of the genome covered by reads (sometimes also called breadth of coverage).

A high coverage in shotgun sequencing is desired because it can overcome errors in base calling and assembly. The subject of DNA sequencing theory addresses the relationships of such quantities. **FastQ**: Each sequence requires at least 4 lines:

1. The first line is the sequence header which starts with an '@'

- 2. The second line is the sequence.
- 3.The third line starts with '+'
- 4. The fourth line are the quality scores

HW-ST911	the unique instrument name
111	the run id
C0N4WACXX	the flowcell id
5	flowcell lane
1101	tile number within the flowcell lane
2249	'x'-coordinate of the cluster within the tile
2216	'y'-coordinate of the cluster within the tile
1	the member of a pair, 1 or 2 (paired-end or mate-pair reads only)
Y	Y if the read is filtered, N otherwise
18	0 when none of the control bits are on
TTAGGC, CGATO	c index sequence

FastA: Each sequence consists of at least two lines:

1. The first is the sequence header, which always starts with a '>'

 Everything from the beginning '>' to the first whitespace is considered the sequence identifier. Everything after that is considered the sequence description (this can be metadata, machine serial number, read orientation, etc.)

2. The sequence itself

Note that the sequence can span multiple lines, depending on the length of the sequence.

Studying a genomic sequence

- Bacterial genome lenght approx. 3-5 x 10⁶ base pairs
- Coverage (or depth) in DNA sequencing is the number of unique reads that include a given nucleotide in the reconstructed sequence. Deep sequencing refers to the general concept of aiming for high number of unique reads of each region of a sequence.
- Even though the sequencing accuracy for each individual nucleotide is very high, the very large number of nucleotides in the genome means that if an individual genome is only sequenced once, there will be a significant number of sequencing errors. Furthermore, many positions in a genome contain rare <u>single-nucleotide polymorphisms</u> (SNPs). Hence to distinguish between sequencing errors and true SNPs, it is necessary to increase the sequencing accuracy even further by sequencing individual genomes a large number of times
- The term "ultra-deep" can refer to higher coverage (>100-fold), which allows for detection of sequence variants in mixed populations



De novo genome assembly

1-Informatic tools normally use FastQ files to produce **FastA files** 2-GapClosing to produce Longer contigs or scaffolds (long reads) PCR-based closure 3-on line annotation

Assembler



short reads and long reads



Studying genome content

- De novo sequencing
- Comparative analysis with reference genomes
- Identification of peculiar genes

DATABASES

Genome annotation



Functional classes of the proteins

- Transporters
- Energy metabolism
- Biosynthesis
 - Amino acids, lipids, nucleotides
- Cell cycle
- Virulence
- Phages

Fundamental protein domains <u>www.ncbi.nlm.nih.gov/COG/</u>

Prediction of the function of a protein deduced from a DNA sequence on the basis of its functional domains



The SMART diagram above represents a summary of the results shown balow. Domains with acores less significant than established cutoffs are not shown in the diagram. Features are also not shown when two or more occupy the same piece of sequence; the priority for display is given by SMART > PRAM > PROSPERO repeats > Signal peptide > Transmembrane > Solied coil > Unstructured regions > Low complexity. In either case, features not shown in the above diagram are marked as 'overlap' in the right side table below.

Confidently predicted domains, repeats, motifs and features:					
Name	Start 🛦	End	E-value		
CUB	9	130	3.63e-31		
EGF_CA	131	172	2.37e-7		
СИВ	175	290	9.8e-28		
CCP	294	354	1.04e-8		
CCP	359	421	1.3e-9		
Tryp_SPc	437	675	4.36e-75		

Features NOT shown in the diagram: 🕢

Name	Start .	End	E-value	Reason
END	134	153	276	threshold
EGF	134	172	0.0118	threshold
Pfam:FXa_i	135	171	1.9e-8	overlap
Pfam:HRM	138	178	14000	overlap
PostSET	139	152	955	threshold
Amb V all	140	176	13200	threshold

Click on a row to highlight the feature in the diagram above. Click the feature name for more informat



Nucleic Acids Res, Volume 46, Issue D1, 4 January 2018, Pages D493–D496 20 years of the SMART protein domain annotation resource

https://doi.org/10.1093/nar/gkx922

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Subsystem Information

Subsystem Statistics Features in Subsystems



Example of genomics

Acinetobacter

Barbe V, Vallenet D, Fonknechten N, Kreimeyer A, Oztas S, Labarre L, Cruveiller S, Robert C, Duprat S, Wincker P, Ornston LN, Weissenbach J, Marlière P, Cohen GN, Médigue C.

Unique features revealed by the genome sequence of Acinetobacter sp. ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res. 2004 Oct 28;32(19):5766-79. doi: 10.1093/nar/gkh910.

Acinetobacter baylyi



Acinetobacter baumannii

Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN, Gerstein M, Snyder M. New insights into Acinetobacter baumannii pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes & development. 2007 Mar 1;21(5):601-14. **ATCC strain**

- A. baumannii ACICU contains a single circular chromosome of 3,904,116 bp and two plasmids (pACICU1 and pACICU2) of 28,279 and 64,366 bp, respectively; 3,758 genes were annotated in the ACICU chromosome, including 3,670 predicted protein-encoding CDSs, 64 tRNA genes, and 8 rRNA operons.
- Nearly 70% of the CDSs (n = 2,670) were assigned to a COG functional category; several genes belonged to more than one COG class.

Genome annotation



- The A. baumannii ACICU genome was initially compared with the unique genomes of Acinetobacter available, A. baumannii ATCC 17978 and Acinetobacter baylyi ADP1, with the aim of identifying novel genes related to virulence and drug resistance.
- Genome comparison showed 86.4% synteny with A. baumannii ATCC 17978 and 14.8% synteny with A. baylyi ADP1
- For many COG classes, the number of CDSs identified in ACICU largely exceeds the number identified in ATCC 17978, since in the latter strain only 60.1% of the genes were assigned to a COG class

36 putative alien islands (pAs) were detected in the ACICU genome; 24 of these had previously been described in the ATCC 17978 genome, 4 are proposed here for the first time and are present in both ATCC 17978 and ACICU, and 8 are unique to the ACICU genome.

Acinetobacter spp. synthenia



- ACICU also contains 14 ISs in the chromosome, including 7 ISAba125 elements, 4 ISAba2 elements, 2 IS26 elements, and 1 ISPu12 element, and 11 on plasmids, including 3 ISAba3 elements, 3 IS26 elements, 4 ISAba2 elements, and 1 ISAba125 element.
- The chromosome is composed of 0.38% short repetitive mini- and microsatellite DNA sequences






Other Acinetobacter baumannii

Iacono M, Villa L, Fortini D, Bordoni R, Imperi F, Bonnal RJ, Sicheritz-Ponten T, De Bellis G, Visca P, Cassone A, Carattoli A. Whole-genome pyrosequencing of an epidemic multidrug-resistant Acinetobacter baumannii strain belonging to the European clone II group. Antimicrobial agents and chemotherapy. 2008
Jul;52(7):2616-25. Clinical carbapenem resistant ACICU strain
Received 21 December 2007 Revision received 28 February 2008 Accepted 8
April 2008 Published 1 July 2008

Vallenet D, Nordmann P, Barbe V, Poirel L, Mangenot S, Bataille E, et al. (2008) Comparative Analysis of Acinetobacters: Three Genomes for Three Lifestyles. PLoS ONE 3(3): e1805, March 19 2008. **AYE and SDF Received:** September 20, 2007; **Accepted:** February 9, 2008; **Published:** March 19, 2008

NEWS & ANALYSIS

GENOME WATCH

Opportunity knocks

Helena Seth-Smith & Alan Walker

This month's Genome Watch examines recent genome papers that provide insight into opportunistic pathogenesis.

Acinetobacter baumannii is emerging as an opportunistic pathogen that primarily infects immunocompromised patients in hospitals and particularly those in intensive-care units. The main clinical outcomes of infection (pneumonia, meningitis, bacteraemia and urinary-tract infections) are compounded by the problem of multidrug resistance. The natural reservoir of A. baumannii is unknown, but it can persist in hospital environments and is commonly found on the skin. A. baumannii has also been isolated from body lice, which suggests that it might use these insects as vectors¹.

Four strains of A. baumannii were recently sequenced: <u>A. baumannii ATCC 17978</u>, a historic strain from 1951 that was implicated in fatal meningitis in a 4-month-old baby; <u>A. baumannii SDE</u> which was isolated from a human-body louse in France; <u>A. baumannii AYE</u>, which was isolated in 2001 during a nationwide outbreak in France; and <u>A. baumannii</u> ACICU, which was isolated from the cerebrospinal fluid of a patient during an outbreak in flaly in 2005 (REFS 2–4).

One of the most striking observations is the amount of apparently horizontally acquired DNA that is present in A. baumannii genomes. Members of the Acinetobacter genus can take up foreign DNA and incorporate it into their own genomes. A. baumannii ATCC 17978 carries 28 putative alien islands, which account for more than 17% of the predicted coding sequences (CDSs). The more recent strain A. baumannii ACICU possesses an additional 8 putative alien islands. The louse-associated strain A. baumannii SDF does not contain intact copies of all the genes that are necessary for natural transformation and, perhaps as a result, it contains fewer strain-specific CDSs than



A. baumannii AYE. However, it contains 428 copies of insertion sequences, a massive expansion compared with A. baumannii AYE (33) and A. baumannii ACICU (14). This has resulted in a greater proportion of pseudogenes (more than 9%) and associated deletions, reducing the overall genome size (3.4 Mb compared with 3.9 Mb in the other strains) and perhaps restricting its host range.

Both A. baumannii ACICU and A. baumannii ATCC 17978 contain two plasmids, whereas A. baumannii SDF has three plasmids and A. baumannii AYE has 4 plasmids. Plasmid pACICU1 from A. baumannii ACICU might encode carbapenem resistance, but none of the other plasmids contains obvious resistance or virulence markers.

Generally considered a low-virulence species, candidate virulence factors have proved hard to identify. Many of the putative alien islands carry potential virulence genes, including type IV secretion systems, siderophores and haemolysins/haemagglutinins. Screens of transposon mutants of A. baumannii ATCC 17978 in both <u>Caenorhabditis elegans</u> and <u>Dictyostelium discoideum</u> identified several genes that are involved in virulence. However,

some of these were strain specific. Surface structures may be important in the ability of *A. baumannii* to form biofilms, which could aid the survival of this organism in hospital environments.

Glucokinase is absent from the sequenced genomes, which means that the strains cannot perform the first steps of glycolysis: an inability to grow on glucose as a sole carbon source has long been used to identify *Acinetobacter* species³. However, *A. baumannii* can catabolize a wide range of alternative carbon sources: *A. baumannii* ACICU seems to have the ability to use benzoate, citrate and glycerol, among other sources, and *A. baumannii* AYE has a substantial catabolic repertoire, including several uncharacterized oxygenases.

A. baumannii is intrinsically resistant to many antibiotics, putatively owing to the presence of many outer-membrane proteins and efflux pumps. Even A. baumannii ATCC 17978, which was isolated in 1951 and therefore had not been exposed to many antibiotics, possesse several efflux pumps, including 19 resistance-nodulation-division (RND) transporters, 3 major facilitator superfamily (MFS)

Four strains of A. baumannii were recently sequenced: A. baumannii ATCC 17978, an historic strain from 1951 that was implicated in fatal meningitis in a 4-month-old baby; A. baumannii SDF, which was isolated from a human-body louse in France; A. baumannii AYE, which was isolated in 2001 during a nationwide outbreak in France; and A. baumannii ACICU, which was isolated from the cerebrospinal fluid of a patient during an outbreak in Italy in 2005 (Refs 2,3,4).

Nature Reviews Microbiology 6, pages 652–653 (2008)



Coregenome Pangenome

- Coregenome represents the genes present in all strains of a species = indispensable genome
- The accessory or flexible genome or dispensable genome: represents the genes that are present in some strains but not in the whole species
- The pangenome is the pool of all genes accessible to a species, both those of the coregenome and the accessory genes
- The ultimate goal is to understand the phenotype of a species, but also the phenotypic differences between isolates of the same species



Core genome pan genome calculation

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ACICU

ACICU

The genomics of Acinetobacter baumannii: insights into genome plasticity, antimicrobial resistance and pathogenicity. **Imperi** et al IUBMB Life. 2011 Dec;63(12):1068-74



A whole genome phylogeny of 136 sequenced genomes in the genus Acinetobacter based on umannii annii 908-14 Imannii 200 baumanin annii 1656. A. bauma , baumannii baum A. baumannii ABNIHA baum A. baumann , baumannii ABN **SNPs** Iannii ACICU



^{nannii T}CDC 48-715

nnii W6976

ⁿⁿⁱⁱ 6014059

nii TG

Imannii ABNIH2

Sahl et al. (2013) Evolution of a Pathogen: A Comparative Genomics Analysis Identifies a Genetic Pathway to Pathogenesis in Acinetobacter. PLoS ONE 8(1): e54287.

identification of genes in the genome



Lipopolysaccharide (LPS)

- Endotoxin or Pyrogen
 - Fever causing
 - Toxin nomenclature
 - Endo- part of bacteria
 - Exo- excreted into environment
- Structure
 - Lipid A
 - Polysaccharide
 - O Antigen of E. coli, Salmonella
- G- bacteria only
 - Alcohol/Acetone removes







2-keto-3deoptonatec



Architecture of a channel-forming O-antigen polysaccharide ABC transporter Bi et al. Nature. 2018 Jan 18; 553(7688): 361–365.



Serotyping



In silico Serotyping







DebRoy C et al. PLoS One. 2016 Jan 29;11(1):e0147434



Comparison of O-Antigen Gene Clusters of All O-Serogroups of Escherichia coli and Proposal for Adopting a New Nomenclature for O-Typing. **DebRoy** C et al. PLoS One. 2016 Jan 29;11(1):e0147434





The EMBD Journal (2008) 27, 2211–2210 | © 2008 European Molecular Biology Organization | Some Rights Reserved (261 4125/08 serve embolscimation)



New EMBO Member's Review

Architectures and biogenesis of non-flagellar protein appendages in Gram-negative bacteria

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Remi Fronzes, Han Remaut and Gabriel Waksman* way: chaperone-usher (CU) ptil, curli, type IV ptil, type III secretion needle and type IV secretion pili (Figure 1).

Pili and fimbriae

Non-flagellar protein appendages in Gram-negative bacteria

R Fronzes et al



fimbriae



MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Dec. 2007, p. 551–575 1092-2172/07/508.00+0 doi:10.1128/MMBB.00014-07 Copyright 0.2007, American Society for Microbiology. All Rights Reserved.

olution of the Chaperone/Usher Assembly Pathway: Fimbrial Classification Goes Greek†

Sean-Paul Nuccio and Andreas J. Bäumler*

chaperone usher Tip adhesin







Escherichia coli

K12-MG1655 (no pathogenic) Enterohemorrhagic EHEC (O157:H7, STX) Uropathogenic UPEC (pili P) Enteropathogenic EPEC (T3SS) Enterotoxigenic ETEC (LT e adesine) Enteroaggregative EAEC (fimbriae)



35 Kb locus of enterocyte effacement (LEE); bundle-forming pilus gene (*bfp*); Shiga toxin genes (stx_1 , stx_2 ,); Heat-Labile toxin (LT); Heat-Stable toxin (ST); colonization factors (CFs); acquired fimbriae that enhance adherence (Afa/Dr); pAA plasmid; pINV plasmid; chromosomal pathogenicity islands (PAIs) Croxen et al. CMR 2013



Circa 3.600.000 risultati (0,40 secondi)



https://www.ncbi.nlm.nih.gov>...

Microbial Genomes - NCBI

Microbial Genomes resource presents public data from prokaryotic genome sequencing projects. Prokaryotes are the earliest forms of life, appearing on earth ...

Articoli accademici per microbial genomes ncbi

Update on RefSeq **microbial genomes** resources - Tatusova - Citato da 144 The integrated **microbial genomes** (IMG) system - Markowitz - Citato da 459 ... **microbial genomes** database: new representation and ... - Tatusova - Citato da 487



Microbial Genome Resources - NCBI

Microbial Genomes Resources presents public data from prokaryotic genome sequencing projects. The sequence collection contains data from finished genomes as ...

Genome

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Using Microbial Genomes

Browse microbial genomes

Download/FTP Refseq Archaea genomes

Download/FTP Refseq Bacteria genomes

Microbial Genomes

Microbial Genomes resource presents public data from prokaryotic genome sequencing projects. Prokaryotes are the earliest forms of life, appearing on earth 4 billion years ago. The Prokaryotes include the Archaea, which include inhabitants of some of the most extreme environments on the planet, and the Bacteria, which include both important pathogens and producers of fermented food, antibiotics, and vitamins.

Annotation Tools						
Prokaryotic Annotation Pipeline	Microbi					
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Glimmer						
ORF finder						

Analysis Tools

Microbial Genomes BLAST

Search

Genome Submission

RegisterBioproject

Submit SRA data

Submit a genome

Submission Guide

Related Resources
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Genome
BioProject
BioSample

Contact and Outreach

NCBI Handbook

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1	'Brassica napus' phytoplasma	Bacteria;Terrabacteria group;Tenericutes	TW1	SAMN09083457	PRJNA46439	1GCA_003181115.1	۲	0.74359	8 27.20	
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Genome > Genome Information by Organism

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#	Organism Name	Organism Groups	\$\$ Strain	BioSample	BioProjec	\$ Assembly	🗘 Leve 🌲	Size	\$ GC%	
1	Escherichia coli str. K-12 substr. MG1655	Bacteria;Proteobacteria;Ga	K-12 substr. MG1655	SAMN02604091	PRJNA225	GCA_000005845.2	•	4.64	50.80	chromosome
2	Escherichia coli O157:H7 str. Sakai	Bacteria;Proteobacteria;Ga	Sakai substr. RIMD 0509952	SAMN01911278	PRJNA226	GCA_000008865.2	•	5.59	50.45	chromosome plasmid pOS plasmid pO1
3	Escherichia coli	Bacteria;Proteobacteria;Ga	136	SAMN08773043	PRJNA445267	GCA_005221645.1	•	5.56	50.55	chromosome plasmid pTA1 plasmid pTA1
4	Escherichia coli	Bacteria;Proteobacteria;Ga	140	SAMN08773047	PRJNA44526	GCA_005221925.1	•	5.56	50.55	chromosome plasmid pTA plasmid pTA
5	Escherichia coli	Bacteria;Proteobacteria;Ga	117	SAMN08773029	PRJNA445267	GCA_005221825.1	•	5.56	50.55	chromosome plasmid pTA1 plasmid pTA1
6	Escherichia coli	Bacteria;Proteobacteria;Ga	120	SAMN08773032	PRJNA445267	GCA_005221805.1	•	5.56	50.55	chromosome plasmid pTA1 plasmid pTA1
7	Escherichia coli	Bacteria;Proteobacteria;Ga	138	SAMN08773045	PRJNA445267	GCA_005221965.1	•	5.56	50.55	chromosome plasmid pTA plasmid pTA
8	Escherichia coli	Bacteria;Proteobacteria;Ga	121	SAMN08773033	PRJNA44526	GCA_005222025.1	•	5.56	50.55	chromosome plasmid pTA plasmid pTA

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	Escherichia coli str. K-12 substr. MG1655, complete genome.						
VERSION	NC 000913.3		Related information				
DBLINK	BioProject: PRJNA57779		Assembly				
	BioSample: <u>SAMN02604091</u>		BioProject				
KEYWORDS	RefSeq. Escherichia coli str. K-12 substr. MG1655		BioSample				
ORGANISM	Escherichia coli str. K-12 substr. MG1655		B				
	Bacteria; Pseudomonadota; Gammaproteobacteria; Enterobacterales;		Protein				
	Enterobacteriaceae; Escherichia.		PubMed				
AUTHORS	1 (bases 1 to 4641652) Rilev.M., Abe.T., Arnaud.M.B., Berlvn.M.K., Blattner.F.R.,		Taxonomy				
	Chaudhuri,R.R., Glasner,J.D., Horiuchi,T., Keseler,I.M., Kosuge,T.,		Components (Core)				
	Mori,H., Perna,N.T., Plunkett,G. III, Rudd,K.E., Serres,M.H.,						
TITLE	Inomas,G.H., Inomson,N.K., Wisnart,D. and Wanner,B.L. Escherichia coli K-12: a cooperatively developed annotation		Full text in PMC				
	snapshot2005		Gene				
JOURNAL	Nucleic Acids Res. 34 (1), 1-9 (2006)		Genome				
PUBMED	16397293 Publication Status: Online-Only		Hanfard Ore D. 1. C				
REFERENCE	2 (bases 1 to 4641652)		identical GenBank Sequence				
AUTHORS	Hayashi,K., Morooka,N., Yamamoto,Y., Fujita,K., Isono,K., Choi,S.,		PubMed (Weighted)				
TTTLE	Ohtsubo,E., Baba,T., Wanner,B.L., Mori,H. and Horiuchi,T.		Reference Genome BioProject				
ITILE	nighty accurate genome sequences of escherichia coli K-12 strains MG1655 and W3110		Poprocontativo Conomo PicProj	act			
JOURNAL	Mol. Syst. Biol. 2, 2006 (2006)		Representative Genome DioProj	501			
PUBMED	16738553						
REFERENCE	3 (bases 1 to 4641652)						

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>NC 000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACGAGT TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT GACGGGACTCGCCGCCGCCCAGCCGGGGTTCCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAA AACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA GGCAGTGGGGCATTACCTCGAATCTACCGTCGATATTGCTGAGTCCACCCGCCGTATTGCGGCAAGCCGCATTCCGGCTG ATCACATGGTGCTGATGGCAGGTTTCACCGCCGGTAATGAAAAAGGCGAACTGGTGGTGCTTGGACGCAACGGTTCCGAC CGACCCGCGTCAGGTGCCCGATGCGAGGTTGTTGAAGTCGATGTCCTACCAGGAAGCGATGGAGCTTTCCTACTTCGGCG CTAAAGTTCTTCACCCCGCACCATTACCCCCATCGCCCAGTTCCAGATCCCTTGCCTGATTAAAAATACCGGAAATCCT CAAGCACCAGGTACGCTCATTGGTGCCAGCCGTGATGAAGACGAATTACCGGTCAAGGGCATTTCCAATCTGAATAACAT GGCAATGTTCAGCGTTTCTGGTCCGGGGATGAAAGGGATGGTCGGCATGGCGGCGCGCGTCTTTGCAGCGATGTCACGCG CCCGTATTTCCGTGGTGCTGATTACGCAATCATCTTCCGAATACAGCATCAGTTTCTGCGTTCCACAAAGCGACTGTGTG CGAGCTGAACGGGCAATGCAGGAAGAGTTCTACCTGGAACTGAAAGAAGGCTTACTGGAGCCGCTGGCAGTGACGGAACG GCTGGCCATTATCTCGGTGGTAGGTGATGGTATGCGCACCTTGCGTGGGATCTCGGCGAAATTCTTTGCCGCACTGGCCC GCGCCAATATCAACATTGTCGCCATTGCTCAGGGATCTTCTGAACGCTCAATCTCTGTCGTGGTAAATAACGATGATGCG ACCACTGGCGTGCGCGTTACTCATCAGATGCTGTTCAATACCGATCAGGTTATCGAAGTGTTTGTGATTGGCGTCGGTGG GTGTTGCCAACTCGAAGGCTCTGCTCACCAATGTACATGGCCTTAATCTGGAAAACTGGCAGGAAGAACTGGCGCAAGCC AAAGAGCCGTTTAATCTCGGGCGCTTAATTCGCCTCGTGAAAGAATATCATCTGCTGAACCCGGTCATTGTTGACTGCAC TTCCAGCCAGGCAGTGGCGGATCAATATGCCGACTTCCTGCGCGAAGGTTTCCACGTTGTCACGCCGAACAAAAGGCCA ACACCTCGTCGATGGATTACTACCATCAGTTGCGTTATGCGGCGGAAAAATCGCGGCGTAAATTCCTCTATGACACCAAC GTTGGGGCTGGATTACCGGTTATTGAGAACCTGCAAAATCTGCTCAATGCAGGTGATGAATTGATGAAGTTCTCCGGCAT TCTTTCTGGTTCGCTTTCTTATATCTTCGGCAAGTTAGACGAAGGCATGAGTTTCTCCGAGGCGACCACGCTGGCGCGGG GAAACGGGACGTGAACTGGAGCTGGCGGATATTGAAATTGAACCTGTGCCCGCAGAGTTTAACGCCGAGGGTGATGT TTTTGCGCTATGTTGGCAATATTGATGAAGATGGCGTCTGCCGCGTGAAGATGCCGAAGTGGATGGTAATGATCCGCTG TTCAAAGTGAAAAATGGCGAAAACGCCCTGGCCTTCTATAGCCACTATTATCAGCCGCTGCCGTTGGTACTGCGCGGATA TGGTGCGGGCAATGACGTTACAGCTGCCGGTGTCTTTGCTGATCTGCTACGTACCTCTCATGGAAGTTAGGAGTCTGAC ATGGTTAAAGTTTATGCCCCGGCTTCCAGTGCCAATATGAGCGTCGGGGTTTGATGTGCTCGGGGCGGCGGCGGTGACACCTGT TGATGGTGCATTGCTCGGAGATGTAGTCACGGTTGAGGCGGCAGAGACATTCAGTCTCAACAACCTCGGACGCTTTGCCG

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>NC 002695.2 Escherichia coli 0157:H7 str. Sakai DNA, complete genome AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTCTCTGACAGCAGCTTCTGAACTG GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCACCACCATCACC ATTACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCT TTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACG TCACCAACCACCTGGTGGCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGT ATTTTTGCCGAACTTCTGACGGGACTCGCCGCCGCCAGCCGGGATTCCCGCTGGCGCAATTGAAAACTTTCGTCGACCA GGAATTTGCCCAAATAAAACATGTCCTGCATGGCATTAGTTTGTTAGGGCAGTGCCCGGATAGCATTAACGCTGCGCTGA TTTGCCGTGGCGAGAAAATGTCGATCGCCATTATGGCCGGCGTATTAGAAGCGCGCGGTCACAACGTTACCGTTATCGAT CCGGTCGAAAAACTGCTGGCAGTGGGGCATTACCTCGAATCTACTGTCGATATTGCAGAGTCCACCCGCCGTATTGCGGC AAGTCGTATTCCGGCTGATCACATGGTGCTGATGGCAGGTTTCACCGCCGGTAATGAAAAAGGCGAACTGGTGGTACTTG GACGGGGTATATACCTGCGACCCGCGTCAGGTGCCCGATGCGAGGTTGTTGAAATCGATGTCCTACCAGGAAGCGATGGA GCTTTCCTACTTCGGCGCTAAAGTTCTTCACCCCCGCACCATTACCCCCATCGCCCAGTTCCAGATCCCTTGCCTGATTA AAAATACCGGAAATCCTCAAGCTCCAGGTACGCTCATTGGTGCCAGTCGTGATGAAGACGAATTACCGGTCAAGGGCATT TGCTGCAATGTCACGCGCCCGTATTTCCGTGGTGCTGATTACGCAATCATCTTCCGAATACAGTATCAGTTTCTGCGTTC CGCAAAGCGACTGTGTGCGAGCTGAACGGGCAATGCAGGAAGAGTTCTACCTGGAACTGAAAGAAGGCTTACTGGAGCCG CTGGCGGTGACGGAACGGCTGGCCATTATCTCGGTGGTAGGTGATGGTATGCGCACCTTGCGTGGGATCTCGGCGAAATT CTTTGCCGCGCTGGCCCGCGCCAATATCAACATTGTCGCTATTGCTCAGGGATCTTCTGAACGCTCAATCTCTGTCGTGG TAAATAACGATGATGCGACCACTGGCGTGCGCGTTACTCATCAGATGCTGTTCAATACCGATCAGGTTATCGAAGTGTTT GTGATTGGCGTCGGTGGCGTTGGCGGTGCGCTGCTGGAGCAACTGAAGCGTCAGCAAAGCTGGTTGAAGAATAAACATAT CGACTTACGTGTCTGCGGTGTTGCTAACTCGAAGGCTCTGCTCACCAATGTGCATGGCCTAAATCTGGAAAACTGGCAGG AAGAACTGGCGCAAGCCAAAGAGCCGTTTAATCTCGGGCGCTTAATTCGCCTCGTGAAAGAATATCATCTGCTGAACCCG GTCATTGTTGACTGCACCTCCAGCCAGGCAGTGGCGGATCAATATGCCGACTTCCTGCGCGAAGGTTTCCACGTTGTCAC GCCGAACAAAAAGGCCAACACCTCGTCGATGGATTACTACCATCTGTTGCGTCATGCGGCTGAAAAATCGCGGCGTAAAT TCCTCTATGACACCAACGTTGGGGCTGGATTACCGGTTATTGAGAACCTGCAAAATCTGCTCAATGCTGGTGATGAATTG ATGAAGTTCTCCGGCATTCTTTCAGGTTCGCTTTCTTATATCTTCGGCAAGTTAGACGAAGGCATGAGTTTCTCCGAGGC GACTACGCTGGCGCGGGAAATGGGTTATACCGAACCGGATCCGCGAGATGATCTTTCTGGTATGGATGTAGCGCGCGTAAAC TATTAATTCTCGCTCGTGAAACGGGACGTGAACTGGAGCTGGCGGATATTGAAATTGAACCTGTGCCCGCAGAGTTT CCGTGATGAAGGAAAAGTTTTGCGCTATGTTGGCAATATTGATGAAGATGGCGTCTGCCGCGTGAAGATTGCCGAAGTGG ATGGTAATGATCCGCTGTTCAAAGTGAAAAATGGCGAAAACGCCCTGGCCTTTTATAGCCACTATTATCAGCCGCTGCCG

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The use of sequencing technologies is currently transforming almost every aspect of biological science. In relation to infectious diseases, the advances are rapidly changing our scientific discoveries, as well as diagnostic and outbreak

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VirulenceFinder 2.0

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			stx1	A	100	948 / 948	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	29249042925851	Shiga toxin 1, subunit A, variant a	<u>EF079675</u>		
			stx1	В	100	270 / 270	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	29246252924894	Shiga toxin 1, subunit B, variant a	<u>AM230663</u>		
			stx2	2A	100	960 / 960	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	12671071268066	Shiga toxin 2, subunit A, variant a	<u>AB048837</u>		
			stx2	2B	100	270 / 270	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	12680781268347	Shiga toxin 2, subunit B, variant a	<u>AE005174</u>		
			tccF	þ	100	1014 / 1014	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	26696882670701	Tir-cytoskeleton coupling protein	<u>AB253537</u>		
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	gad	100	1401 / 1401	NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome	15706451572045	Glutamate decarboxylase	<u>U00096</u>		
	hlyE	100	918 / 918	NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome	12294831230400	Avian E.coli haemolysin	<u>ECU57430</u>		
	iss	98.98	294 / 294	NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome	578600578893	Increased serum survival	<u>CP001509</u>		
	ompT	100	954 / 954	NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome	584680585633	Outer membrane protease (protein protease 7)	<u>AP009048</u>		
	terC	100	714 / 714	NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome	29704202971133	Tellurium ion resistance protein	<u>CP007491</u>		
	terC	99.9	966 / 966	NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome	32385803239545	Tellurium ion resistance protein	<u>MG591698</u>		

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pMLST

Multi Locus Sequence Typing (MLST) from an assembled plasmid or from a set of reads.

cgMLSTFinder

Core genome Multi Locus Sequence Typing (cgMLST) from a set of reads.

KmerFinder

Prediction of bacterial species using a fast K-mer algorithm.

MGE

Identification of mobile genetic elements and their relation to antimicrobial resistance genes and virulence factors.

SpeciesFinder

Prediction of bacterial species using the S16 ribosomal DNA

sequence.

<u>SeroTypeFinder</u>

Prediction of serotypes in total or partial sequenced isolates of E. coli.

SeqSero

SeqSero predicts the Salmonella serotype of either the pre-assembled or raw read sequence data provided to the service.

<u>spaTyper</u>

spaTyper predicts the S. aureus spa type.

<u>FimTyper</u>

FimTyper predicts the E. coli Fim type.

CHTyper

CHTyper predicts the E. coli FimH type and FumC type.

https://cge.cbs.dtu.dk/services/SpeciesFinder/ DAct

Other

MyKMAfinder

MyKMAfinder performs typing or pheno typing using KMA based on a user defined database.

MyDbFinder

MyDbFinder performs typing or pheno typing using blast based on a user defined database.

MyKmerFinder

MyKmerFinder performs typing or pheno typing using Kmers based on a user defined database.

DeHumanizer

The DeHumanizer web-server is a tool for human filtering based on the method described by Zhang et al.

HostPhinder

HostPhinder identifies the bacterial host of a query phage genome based on its genomic similarity to a database of phage genomes with known host.

MetaPhinder

MetaPhinder: Identifying Bacteriophage Sequences in Metagenomic Data Sets.

Plain text sequence.fasta

Home	Services	Instructions	Output	Article abstract
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Center for Genomic Epidemiology

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SerotypeFinder-2.0 Server - Results

Database(s): H_type,O_type

	Database for H type genes									
Gene	Serotype	Identity	Template / HSP length	Contig	Position in contig	Accession number				
fliC	H7	100	1758 / 1758	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	26245162626273	AF228487				

				Database for O type genes		
Gene	ne Serotype Identity Template / HSP length		/pe Identity Template / Contig		Position in contig	Accession number
wzy	O157	100	1185 / 1185	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	27854472786631	<u>JH953200</u>
wzx	O157	100	1392 / 1392	NC_002695.2 Escherichia coli O157:H7 str. Sakai DNA, complete genome	27833542784745	<u>JH959508</u>

extended output

Results as text Results tsv Hits in genome seqs Serotype gene sequences

Selected %ID threshold: 85 %

Selected minimum length: 60 %

Input Files: sequence.fasta

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https://usegalaxy.eu/

🗧 Galaxy Europe	☆ Workflow Visualize	ta 🕶 Help 👻 Login or Register 🖅 🌐	
Tools	COVID-19 Research!		History
search tools	Want to learn the best practices for the analysis of SARS-CoV-2 data using Galaxy? Visit the Galaxy SARS-CoV-2 portal. We mirror all public		search datasets
	SARS-CoV-2 data from ENA in a Galaxy data library for your convenie materials. Please check our recent activities for more details.	ence. The Galaxy community has created COVID-19 dedicated training	Unnamed history
1 Upload Data	If you need help submitting your data to public archives, like ENA, plea	ase get in touch. We will support you in sharing your data.	(empty)
Get Data			
Send Data	"Anyone, anywhere in the world should have free, unhindered access to	This history is empty. Y your own data or get an external source	
Collection Operations	across the spectrum of human understanding." – Prof. Stephen Hawking		
GENERAL TEXT TOOLS	News	Events	
Text Manipulation	Oct 12, 2021		
Filter and Sort	BY-COVID: A new EU project for pandemic	avancée de séquences	
Join, Subtract and Group	preparedness	Oct 20, 2021 ∰	
GENOMIC FILE MANIPULATION	Oct 12, 2021 UseGalaxy.eu Use Case: cellular specification, differentiation and morphogenesis of the mucociliary epithelium Oct 11, 2021 UseGalaxy.eu Use Case: microRNAs in heart disease		
Convert Formats			
FASTA/FASTQ		Oct 20, 2021 ∰ [7] International Galaxy Proteomics Meeting	
Quality Control		Series Oct 21, 2021	
SAM/BAM			
BED	Oct 8, 2021	∰ 🗗 Galaxy Paper Cuts	
VCF/BCF	A proteomics sample metadata representation for multiomics integration and big data analysis	Oct 22, 2021	
Nanopore		image analysis possible	
COMMON GENOMICS TOOLS	New brochure from ELIXIR Germany	Oct 22, 2021	
Operate on Genomic Intervals	Oct 5, 2021		
Fetch Sequences / Alignments	UseGalaxy.eu FTP Server Update		
GENOMICS ANALYSIS			
Annotation	Currently Runni	ing and Queued Jobs	
Multiple Alignments	2 K 1.50 K		
Assembly	1 K 500		

Prokka prokaryotic genome annotation in Galaxi Europe

🗲 Galaxy Europe	🛠 Workflow Visualize 🕶 Shared Data 🕶 Help 👻 User 👻 📻 🏢	Using 13%
Tools	Prokka Prokaryotic genome annotation (Galaxy Version 1.14.6+galaxy0)	▲ History 2 + □ ↓ search datasets 2 2
Lyboad Data Show Sections Prokka Prokaryotic genome annotation Roary the pangenome pipeline - Quickly generate a core gene alignment from gff3 files	Contigs to annotate Image: Description of the second se	0323 (empty) I This history is empty. You can load your own data or get data from an external source
WORKFLOWS All workflows	I (increment) GFF version 3 (gffver) Force GenBank/ENA/DDJB compliance No Equivalent toaddgenesmincontiglen 200centre Prokka (or other centre specified below) (compliant) Add 'gene' features for each 'CDS' feature (addgenes) Image: Sequencing centre ID (centre) Genus name	
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https://rast.nmpdr.org/



Rapid Annotation using Subsystem Technology version 2.0

The NMPDR, SEED-based, prokaryotic genome annotation service. For more information about The SEED please visit <u>theSEED.org.</u>

»Tutorials >Help

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Info: RAST Access Problems

Click here for instructions on how to resolve several of the most common problems accessing RAST or your RAST data.

Comand-Line API "301 Permanently Moved" Errors

Click here for instructions on how to resolve "301 Permanently Moved" errors when using the RAST batch command-line interface.

To monitor RAST's load and view other news and statistics for RAST and the SEED, please visit "The Daily SEED."

Welcome to RAST

RAST Job Load, last 24 hours





What is RAST?

RAST (Rapid Annotation using Subsystem Technology) is a fully-automated service for annotating complete or nearly complete bacterial and archaeal genomes. It provides high quality genome annotations for these genomes across the whole phylogenetic tree.

We have a number of presentations and tutorials available:

- Registering for RAST
- <u>The IRIS/Automated-Assembly/RASTtk Workshop Presentations and Tutorials</u>
- <u>The SEED/"Classic-RAST" Workshop presentations and Tutorials</u>
- <u>Downloading and installing the RASTtk Toolkit</u>
- Downloading and installing the myRAST Toolkit
- The RAST batch submission interface (a part of myRAST)
- Making manual improvements to RAST-annotated genomes (first tutorial). This is a powerpoint presentation; bring it up in slide-show mode and click through to see the animations and movies.
- Making manual improvements to RAST-annotated genomes (second tutorial). This is a second tutorial on the topic of manually improving RAST annotations; it is also a powerpoint presentation with animations.

As the number of more or less complete bacterial and archaeal genome sequences is constantly rising, the need for high quality automated initial annotations is rising with it. In response to numerous requests for a SEED-quality automated annotation service, we provide RAST as a free service to the community. It leverages the data and procedures established within the <u>SEED framework</u> to provide automated high quality gene calling and functional annotation. RAST supports both the automated annotation of high quality gene calling and functional. RAST supports both the automated annotation of high quality gene calling and functional.

Please note that while the SEED environment and SEED data structures (most prominently FIGfams) are used to compute the automatic annotations, the data is NOT added into the SEED automatically. Users can however request inclusion of a their genome in the SEED. Once annotation is completed, genomes can be downloaded in a variety of formats or viewed online. The genome annotation provided does include a mapping of genes to subsystems and a

https://youtu.be/4H-L1DVD3z8

MLST video in Galaxy

https://youtu.be/MhIZ6llaAac

NCBI Minute: Use Web RAPT to Assemble and Annotate Prokaryotic Genomes