



Sharing sequence data to enable collective action against AMR

Pimlapas Leekitcharoenphon (Shinny) Research Group of Genomic Epidemiology, DTU-Food

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens and Genomics European Union Reference Laboratory for Antimicrobial Resistance (EURL-AMR)

> pile@food.dtu.dk @ShinnyPimlapas $f(x+\Delta x) = \sum_{i=0}^{\infty} \frac{(\Delta x)^{i}}{i!} f^{(i)}(x)$

EURL-AR Training course 2019



Topics

- Submitting data via ENA
- Sharing data via ENA private hub
- Accessing sequence data through ENA private hub
- Accessing WGS results through ENA private hub



Data storage & Access



National Food Institute, Technical University of Denmark

Data storage & Access

European Bioinformatics Institute (EBI)

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Search results for **PRJEB22091**

Showing 15 results out of 129 in All results

Filter your results	Nucleotide sequences (129 results)	
Source All results (129) Nucleotide sequences (129)	PRJEB22091 Danmap 2016	Related data Source: Study ID: PRJEB22091

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Select columns

Showing results 1 - 10 of 127 results

Study accession	Sample accession	Secondary sample accession	Experiment accession	Run accession	Tax ID	Scientific name	Instrument model	Library layout	FASTQ files (FTP)	FASTQ files (Galaxy)	Submitted files (FTP)	Submitted files (Galaxy)	NCBI SRA file (FTP)	NCBI SRA file (Galaxy)	CRAM Index files (FTP)	CRAM Index files (Galaxy)
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Submitting data in ENA

- Manual and automated
- Use Webin (interactive web submission system) for new sequencing projects, assembled sequences and annotation
- For other types of data, different channels are available (e.g. datasubs@ebi.ac.uk, FTP, RESTful web-based service)
- www.ebi.ac.uk/ena/about/submit_and_update

Link to download sequence data for ENA submission

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Submitting data in ENA

- 1) Install a program to connect to FTP
- https://filezilla-project.org

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Home FileZilla Features Screenshots	FileZilla [®] Pro The Best FTP Solution	
Download Documentation FileZilla Pro FileZilla Server	Overview Welcome to the homepage of FileZilla®, the free FTP solution. The <i>FileZilla Client</i> not only supports FTP, but also FTP over TLS (FTPS) and SFTP. It is open source software	
Community Forum Project page Wiki	distributed free of charge under the terms of the GNU General Public License. We are also offering <i>FileZilla Pro</i> , with additional protocol support for WebDAV, Amazon S3, Backblaze B2, Dropbox, Microsoft OneDrive, Google Drive, Microsoft Azure Blob File Storage, and Google Cloud Storage. Last but not least, <i>FileZilla Server</i> is a free open source FTP and FTPS Server.	and
General FAQ Support Contact License Privacy Policy	Support is available through our forums, the wiki and the bug and feature request trackers. In addition, you will find documentation on how to compile FileZilla and nightly builds for multiple platforms in the development section.	
Trademark Policy Development Source code Nightly builds Translations	Download FileZilla Client All platforms Download FileZilla Server Windows only	
Version history Changelog Issue tracker Other projects	Pick the client if you want to transfer files. Get the server if you want to make files available for others.	
libfilezilla Octochess Sponsors:	 ✓ 2019-08-15 - FileZilla Client 3.44.2 released Bugfixes and minor changes: ✓ MSW: Fixed a crash if using predefined sites through fzdefaults.xml 	
	 ♀ Fixes to protocol selection glitches in the Site Manager ♀ Increase maximum length of response lines when using FTP ♀ 2019-08-09 - FileZilla Client 3.44.1 released 	ON ellAds
	Bugfixes and minor changes: • Fixed a regression introduced in 3.44.0-rc1 breaking support for insecure servers • Reach developer effortiessly throu ad banner with 0	's ugh this Carbon.
WHUK Web Hosting UK	 2019-08-09 - FileZilla Client 3.44.0 released Bugfixes and minor changes: O Fixed export in context menu of Site Manager to handle multiple selected items 	
	♦ 2019-07-29 - FileZilla refocuses on security after participation in EU bug bounty program	



- 2) Connect to FTP
- Host: webin.ebi.ac.uk
- Username: Your own Webin ID
- Password: Your own password for your ENA account. Then click 'Connect'

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Host: webin.ebi.ac.uk Username: Webin-37120 Password: ••••• Port: Quickconnect -	
Status: Connection established, waiting for welcome message Status: Insecure server, it does not support FTP over TLS. Status: Logged in Status: Retrieving directory listing Status: Calculating timezone offset of server Status: Timezone offset of server is 0 seconds. Status: Directory listing of "/" successful	
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• 3) Transfer the genome files to ENA FTP

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04_PanCorePlot	Dire	Project 2019 6 17 6 29 14	Directory	17/
05_PangenomeTree	Dire	Project 2019 6 7 15 10 51	Directory	07/
06_gene_absent_LT2_10Jan11	Dire	Project 2019 6 7 9 8 6	Directory	07/
07_Pathogenicity_Islands_11Jan11	Dire	22b B2 001 fasta az	2 818 734 525 gzin com	23
08_Salmonella_MLST_11Jan11	Dire	ACN/IC6 01052017 fa md5	32 md5-file	20,
9_09_smallRNA	Dire	ACNJC6 curated 20170123 aff md5	32 md5-file	30
100_Ana_13Nov14	Dire	ACNJC6 curated 20170204 embl.md5	32 md5-file	04
101_Dublin_01Dec14	Dire	DTU-shit-2-FAB-P1 S129 L001 R1 001 fasta md5	32 md5-file	19/
102_S_hyicus_02Dec14	Dire	DTU-shit-2-I67_S36 001_R1_001 fasta az md5	32 md5-file	19/
103_Resistome	Dire	DTU-shit-2-167_S36 L001_R2_001 fasta az md5	0 md5-file	19/
104_Kayode_Salmonella_28Jan15	Dire	DTU2013-1821-PR/857-pseudintermedius-Staphylococcus-06241-1 S4 001	32 md5-file	13/
105_Infantis_20Feb15	Dire	DTU2013-1821-PRJ857-pseudintermedius-Staphylococcus-06241-1 S4 L001	32 md5-file	13/
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22 files and 381 directories. Total size: 97,370,680 bytes		151 files and 5 directories. Total size: 60,712,631,420 bytes		
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Submitting data in ENA

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- https://www.ebi.ac.uk/ena/submit/sra/#home

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individual study and sam citing data submitted to E	ple should be registered only once. In add NA.	lition, you will be asked to provide infor	mation about the sequencing libraries	and instruments. Please	quote the study acce	ession number (ERP*) when
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• 7) Set release date, provide title, and abstract

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	ΝΙΛ							
Home Search & Browse	Submit & Updat	e About ENA Contact FAQ					_	_
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Home		New Submission	Studies		Samples	Runs		Analyses
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			Select an exist	ting study or Creat	te a new study			
ou can use this page to s	ubmit a new stu	dy into the European Nucleotide	Archive (ENA).					
lease answer the questio	ns below. Mand	atory fields are denoted by (*).						
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ou want to edit and use th	ne 'Edit ENA link	s' tab.						
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.coli				PLEASE functional	ANSWER WITH YES IF YOU genome annotation.	HAVE ANNOTATION: Locus ta	ag prefixes are only asso	ciated to studies providing
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• 8) 'Select Checklist'

EMBL-EBI		:	Services Researc	h Training A	About us	9
European Nucleotide Archive						
ENA Home Search & Browse Submit & Update About ENA Contact FAQ						
					Contact Help	odesk 🚩 Webin-37120 Logout
Home New Submission	Studies	Samples	Ru	ns		Analyses
Start Study Study	>> Sam	nple >>	Run		~	Finish
 Start building your submission We use checklists to help provide required information in a star You will be guided through the following steps: Selecting a checklist Selecting optional fields in addition to mandatory ones Entering your data directly into this application Alternatively, after selecting the checklist and fields you will be Select Checklist > Upload a submission completed using a template spreadsheet If you have downloaded and filled a template spreadsheet please note that only spreadsheets in tab-delimited text forma spreadsheet as Text (Tab delimited). To do this please see the 	andard format. The able to download a template spre template as a template as a template spre template as a template as a template as a template spre template as a template as a tem	adsheet. You can then enter your da mpleted Spreadsheet button. r .txt extensions). If you edited the s	ta in the spreadsh	eet and upload	it. equivalent)	please save the

• 9) Select 'Pathogens Checklists' and then select 'ENA GMI_MDK:1.1'

Home	Now Submission						
	INCW SUDILISSIU	n Stur	dies	Samples	Runs	Contact Helpdes	Mebin-3712
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larine Checklists	es Micro B3 and Tara Oceans sampl	e checklists					
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 10) You have an option to download metadata as excel template or you just click 'Next' to fill out metadata via website

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1							Contact Hel	odesk <mark>🔤 Webin-37120</mark>
Home		New Submission	Studies		Samples	Runs	3	Analyses
Start 🗸	~	Study ✔	>>	Sample	~	Run	>>	Finish
Please select any a	dditional optio	nal fields. Mandatory and reco	ommended fields are sele	cted by default. You	may add any of the optiona	al fields. You may also	add custom fields.	
Filter fields								
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 11) Add 'title', search 'Escherichia coli' (you have to wait until the system give you organism name to choose), add 'collected_by', add 'collection date'

						Contact Help	desk Webin-37120 Logou
Home	New Submission	Studies	<u> </u>	Samples	Runs	Ύ.	Analyses
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Please complete any field to all samples. This will an samples.	s that you would like to apply ct as a template for all	Template Basic Details Unique Name Prefix: * Title: Description: Organism Details	Ecoli				
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		* Scientific Name: Common Name:	Escherichia coli				
		Collection event information * collected_by:	DTU				() ()
		* collection date:	2019-01-30				
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ontaat Haladaak 🔜 Wahin 27120 Lagou

 12) Add 'country', 'latitude', 'longtitude', 'environmental_sample', host health state', 'host scientific name', 'host associated' and 'isolate'

Home	Ŷ	New Submission	Studies		Samples	Runs		Analyse	IS	
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13) add number of sample. In this case, it is only 1 sample to add (click '+Add')

Home	New Submission	Studies		Samples	Runs		Analyses
Start ✔	>> Study 🗸	~	Sample	»	Run	~	Finish
 Please add samples to the samples can be created the add button + Add 1 samples 1 	he submission. Multiple by increasing the number by	Previous Sample No Basic Details * Unique Name: * Title: Description:	ext Sample >				
		Organism Details If your organism is not for us to request a taxon Id to	ound please go <u>here</u> for your organism.	and email <u>datasubs@eb</u>	i.ac.uk with the required de	tails listed on the	page in order for
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Please submit by clicking Alternatively, download	g the Submit Button. your data as a spreadsheet	* Scientific Name:	Escherichia coli				00
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 14) Add 'Unique Name' for the submitted sample. In this case, try to add 'Ecoli_001'

							Contact Help	
Home	New Su	ubmission	Studies	Ŷ	Samples	Runs	Ύ.	Analyses
Start ✔	»	Study 🛩	~	Sample	>	Run	>>	Finish
Please add samples to the samples can be created the add button	ne submission. Mult by increasing the n	tiple umber by	Previous Sample Nex	t Sample >				
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i 4 1-1 of 1 ▶ 14			* Tax Id: 🕙	562				
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• 15) Click type of submitted files. In this case is 'Two Fastq files (Paired)'

				<u>C</u>	ontact Helpdesk 🔤 Webin-37120 Logout
Home	New Submission	Studies	Samples	Runs	Analyses
Start ✔	>> Study 🗸	>> Sam	le 🖌 🛛 >>	Run >	> Finish
Please provide library, instrument Please select the file format. If you	and data file details by uploading a spi I have files of different types please su	readsheet or by editing the table below. bmit them in seperate submissions.			
CRAM					
BAM					
OSFF					
One Fastq file (Single)					
Two fastq files containing before submission. <i>The fin</i> Complete Genomics PacBio HDF5 Oxford Nanopore Mandatory fields are denoted by (paired reads are submitted for eac est reads must be in the first Fastq	th run. All technical sequences includ	ling adaptor sequences, linker sequences, linker sequences, linker sequences, linker sequences, linker sequence	uences and barcode sequences mu in the same order as in the first i	ust be removed from the reads file.
◆ Download Template Spre	adsheet Vpload Complete	d Spreadsheet V Download Sp	readsheet		
[Sample reference suggestions] (])	Sample reference (*) (1)	nstrument Model (*) 🕕 Library Na	ne 🕕 Library Source (*) 🌘	Library Selection (*)	Library Strategy (*) 🕕 Des
*		÷		•	÷
+					



• 16) Add required metadata (*)

	ownload Template Spreads	heet Vpload Comp	leted Spreadsheet	Download Spreadsheet				
	[Sample reference suggestions]	Sample reference (*) (1)	Instrument Model (*) 🕕	Library Name 🕕	Library Source (*) 🕕	Library Selection (*) (1)	Library Strategy (*) 🕕	Des
×	Ecoli_001 \$	Ecoli_001	Illumina MiSeq 😫		GENOMIC \$	RANDOM \$	WGS 🛟	
•								

Mandatory fields	are denoted by (*).						
trategy (*) 🕕	Design description 🕕	Library construction protocol	Insert Size (*) 🕕	First File Name (*) 🕕	First MD5 checksum 🕕	Second File Name (*)	Second MD5 checksum
+			300	Ecoli_036_R1.fq.gz		Ecoli_036_R2.fq.gz	
							Disable Edit mode



17) MD5 checksum is also required

http://onlinemd5.com

🍯 MD5 & SHA1 Ha	sh Generator For File
Generate and verif	y the MD5/SHA1 checksum of a file without uploading it. Choose File no file selected
	Click to select a file, or drag and drop it here(max: 4GB).
Filename:	No File Selected
File size:	0 Bytes
Checksum type:	OMD5 OSHA1 OSHA-256
File checksum:	
Compare with:	
Process:	
	Compare Pause Stop

MD5 & SHA1 Ha	sh Generator For File
Generate and verify	y the MD5/SHA1 checksum of a file without uploading it. Choose File no file selected
	Click to select a file, or drag and drop it here(max: 4GB).
Filename:	Ecoli_036_R1.fq.gz
File size:	87,524,866 Bytes
Checksum type:	OMD5 OSHA1 OSHA-256
File checksum:	E434CAE6777C250EBE45C0DBC34E8B1C
Compare with:	
Process:	100.00%
	Compare Pause Stop

📕 MD5 & SHA1 Ha	sh Generator For File
Generate and verify	the MD5/SHA1 checksum of a file without uploading it. Choose File no file selected
	Click to select a file, or drag and drop it here(max: 4GB).
Filename:	Ecoli_036_R2.fq.gz
File size:	86,096,901 Bytes
Checksum type:	OMD5 OSHA1 OSHA-256
File checksum:	95C4F0A13B3AF36C9AE0EE570266C253
Compare with:	
Process:	100.00%
	Compare Pause Stop





- 18) The last step is to click 'Submit'. If something goes wrong, the system will give you error message.
- DO NOT click 'Submit' in this exercise

Mandatory fields	are denoted by (*).						
trategy (*) 🕕	Design description 🕕	Library construction protocol	Insert Size (*) 🕕	First File Name (*) 🕕	First MD5 checksum 📵	Second File Name (*)	Second MD5 checksum
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							Disable Edit mode
<< Pre	vious						Submit



Topics

- Submitting data via ENA
- Sharing data via ENA private hub
- Accessing sequence data through ENA private hub
- Accessing WGS results through ENA private hub



ENA private hub

- a private repository for submitting and downloading of public and private genomic and phenotypic data for AMR
- A data provider able to upload data to ENA, share data to the hub and download metadata and sequences
 - Require to have ENA account (Webin ID)
 - <u>https://www.ebi.ac.uk/ena/submit</u>
 - Ask ENA to link your ENA account to the private hub
- A data consumer able to download metadata and sequences
 - No ENA account required



ENA private hub

- Submitting data to ENA (only for data provider)
- Share sequence and metadata in the hub (only for data provider)
- Access metadata
- Download sequences



ENA private hub



- 1) Login to the pathogen website using your ENA account
- https://www.ebi.ac.uk/ena/pathogens/login



Password		
rassword	 	
Login		





 2) Click 'Share'. You will see one the columns is 'dcc_bromhead' which is the name of our EURL hub



Welcome to Pathogens







 3) You can share any of your study by clicking at a button under our hub column (dcc_bromhead)

Choose studies to share with data hubs

Type to filter by study a	ccession or title						
Study Accession	Study Title	dcc_broadbent	dcc_bromhead	dcc_cole	dcc_liszt	dcc_schubert	dcc_strauss
PRJEB14086	reference testing for ENGAGE E.coli (P160095)						
PRJEB18618	EURL reference testing						

• 4) Please allow 24 hours for system to link the shared data to the hub



Topics

- Submitting data via ENA
- Sharing data via ENA private hub
- Accessing sequence data through ENA private hub
- Accessing WGS results through ENA private hub



Accessing metadata in ENA hub

- 1) Search through web portal using 'dcc_bromhead' (ENA hub)
- <u>https://www.ebi.ac.uk/ena/pathogens/login</u>

	Survei	ATH illance, Id		GENS n, Investigation	S on				
Home	Report	Share	Search	Notebooks	Reference	About	Support 🖾	Login	
Log	in								
					Username				
					dcc_bromhead	t			
					Password				





- 2) After login, go to 'Search' tab, select data type e.g. 'read run' or 'analysis'
- 3) On the next few pages, you can refine your search and select fields manually



Welcome to Pathogens













• 4) Specify 'Dcc data only' before performing the search





 5) Search results/reports can be viewed and downloaded. You can download report by clicking at Download report either in JSON or TSV. The TSV format can be imported and viewed in Excel

DATA TYPE QUERY FIELDS DATA FILTERS RESULTS Download report: JSON TSV Type to filter results Analysis Accession Description ERZ390162 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390163 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390164 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390165 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390166 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390167 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390168 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390169 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390170 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) ERZ390171 Antibiotic sensitivity test for ERP015694: reference testing for ENGAGE E.coli (P160095) Showing 1 to 10 of 166 rows. 10 🗘 > Rows per page:

Advanced Search

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Back

New Search



Accessing sequence data in ENA hub



The access to the ENA data hub is given via password protected FTP. Below you find FTP specifications, the folder organization to download the fastq files and instructions to set up FTP access via Mac or Windows.

Specifications

Host: ftp://ftp.dcc-private.ebi.ac.uk

Username: dcc bromhead

Password:

Sequences in FASTQ are under folder: vol1/fastq

Filename 🔨	Fi	
 meta upload vol1 	Filename ERA115 ERA613 ERA625 ERA669 ERA775 ERA786	
	ERA965 ERA984 ERZ390 ERZ480	



Topics

- Submitting data via ENA
- Sharing data via ENA private hub
- Accessing sequence data through ENA private hub
- Accessing WGS results through ENA private hub

WGS results via ENA hub

- 1) Go to pathogen web portal using 'dcc_bromhead' (ENA hub)
- <u>https://www.ebi.ac.uk/ena/pathogens/login</u>

	P Surve	ATH illance, Ic	HOC Ientificatio	GENS n, Investigatio	S on					
Home	Report	Share	Search	Notebooks	Reference	About	Support 🖾	Login	· · · · · · · · · · · · · · · · · · ·	
Log	in									
					Username					
					dcc_bromhead	k				
					Password					
					•••••					





• 2) After login, go to 'Explore' tab



Welcome to Pathogens





• 3) Click 'View' to view report. Click 'Download' to download interactive report



Data Hubs

A data hub is a platform that includes workflows for structured data storage and sharing of sequencing data and its analysis interpretations. For more details please see: https://www.biorxiv.org/content/10.1101/555938v1

To see which Data Hubs and analysis workflows are available currently, please see here.

Notebook reports

The basic view contains a summary of the notebook report and can be viewed in most web browsers. The full view with dynamic controls is currently supported in Firefox, Safari, Chrome, and MS Edge. You can also download the full report (as a zip archive) to view in any browser. After downloading, please extract the zip file and open the index.html file within.

Data Hub	Reports (by date)	View Basic Report	View Full Report	Download Full Report (zip)
dcc_benoit	20190913 (latest)	View	View	Download
dcc_bromhead 📫	20190912 (latest)	View	View	Download

Showing 1 to 2 of 2 rows.

Link to download interactive report for dcc_bromhead

View Demo



• 4) To view interactive report; unzip the file and click 'index.html'

cgMLST_analysis_Sep18			AMR_1.html
dcc_bromhead_20190912	►		AMR_2.html
dcc_bromhead_20190912.zip		1	bromhead_AMR.html
Sharing_sequence_data_EURL_Training_Sep2019			bromhead_datahub.html
			bromhead_primary.html
			bromhead_simplified.html
			datahub 1.html
			datahub 2.html
			datahub 3.html
			datahub 4.html
			datahub map 2014-01.html
			datahub map 2014-03.html
			datahub map 2014-06.html
			datahub map 2014-10.html
			datahub map 2015-01.html
			datahub_map_2015-04.html
			datahub_map_2015-05.html
			datahub_map_2015-10.html
			datahub_map_2016-01.html
			datahub_map_2016-05.html
			datahub_map_2016-06.html
			datahub_map_2017-01.html
			datahub_map_2017-06.html
			datahub_map_all-time.html
			o datahub_map.html
			o index.html
			o primary_1.html
			o primary_2.html
			o primary_3.html
			o primary_4.html
			primary_map_2014-01.html
			primary_map_2014-03.html
			primary_map_2014-06.html
			primary_map_2014-10.html
			primary_map_2015-01.html
			primary_map_2015-04.html
		11	primary_map_2015-05.html











Map shows the samples with different MLST types by submission date

monthly plots are available only after 2014-01-01



		AMF	R data - dcc_k	oromhead d	atahuk	o conter	nt ove	erview			
			This notebook w	as using the data	abase sta	te as of 20	190912				
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	out]	Count		scientific nome							
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Table 2.) MIC / Disk diffusion test's results in the datahub

Thank you for your attention

Pimlapas Leekitcharoenphon (Shinny), PhD

Research Group Genomic Epidemiology WHO Collaborating Centre for Antimicrobial Resistance in Food borne Pathogens and Genomics European Union Reference Laboratory for Antimicrobial Resistance

National Food Institute, Technical University of Denmark

pile@food.dtu.dk

ens WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens and Genomics www.antimicrobialresistance.dk

DTU Food, Technical University of Denmark