PROTOCOL for EURGen-RefLabCap External Quality Assessment 2022

1. Overview and objectives

External Quality assessment schemes (EQAs) are an important tool to assess the performance of laboratories in comparison with other laboratories performing the same type of analysis. In the EURGen-RefLabCap EQA 2022, sequence analysis of FASTA files obtained by whole genome sequencing (WGS) of *E. coli* and *Klebsiella pneumoniae* strains is offered. Participants' results in

relation to bioinformatics analysis for typing and prediction of antimicrobial resistance profiles are assessed.

The main **objective** of this EQA is to test and compare technical and analytical skills for WGS-based carbapenem-resistant *Enterobacterales* (CRE) and colistin-resistant CRE (CCRE) resistome profiling and high-risk clone/plasmid identification at the NRLs. After participation in the EQA/benchmarking exercises, network members will be able to identify strengths and weaknesses in their bioinformatics analysis skills for CRE and CCRE, and they will gain experience that will qualify them to design and execute EQA/benchmarking exercises in their own national networks.

The EQA providers will compare the applied national bioinformatics pipelines used for WGS-based AMR detection with a view to verify that results are compatible with those of ECDC and EFSA joint molecular typing platforms and aligned with EU case definitions and EUCAST guidance documents.

The EURGen-RefLabCap EQA 2022 is coordinated as part of a contract with the European Health and Digital Executive Agency (HaDEA) on behalf of the General Directorate Health and Food Safety (DG SANTE) in close collaboration with European Centre for Disease Prevention and Control (ECDC) (SC 2019 74 01 – Service Contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in priority healthcare associated infections). This contract is carried out jointly by the leads of the contract, National Food Institute, Technical University of Denmark (DTU) and the co-contractor National Reference Laboratory for Antimicrobial Resistance (NRL-AMR), Statens Serum Institut (SSI).

2. Introduction

The current EQA programme focuses on *Escherichia coli* and *Klebsiella pneumoniae* and for each test strain, the provided test material (8 FASTA files in total, see Table 1) includes assembled genomes based on sequencing by Illumina technology (short reads) and assembled genomes based on sequencing by Oxford Nanopore Technology (long reads). Participants are invited to proceed with analysis of either the short read- or the long read-based FASTA files, or may select to perform testing of both types of FASTA files. Alternatively, FASTQ files corresponding to the test material in FASTA file format can be provided upon request to laboratories using bioinformatics pipelines that cannot use FASTA files as input.

Information on the methods used to generate the EURGen-RefLabCap EQA 2022 test sequence data is available in Appendix 1.

Table 1: Codes for test material included in the EURGen-RefLabCap EQA 2022

Bacteria code	Test material code	Based on
EURGen-2022-01	EURGen-2022-01-FASTA-sr	short read sequencing (Illumina)
EURGen-2022-01	EURGen-2022-01-FASTA-lr	long read sequencing (MinIon)
EURGen-2022-02	EURGen-2022-02-FASTA-sr	short read sequencing (Illumina)
EURGen-2022-02	EURGen-2022-02-FASTA-lr	long read sequencing (MinIon)
EURGen-2022-03	EURGen-2022-03-FASTA-sr	short read sequencing (Illumina)
EURGen-2022-03	EURGen-2022-03-FASTA-lr	long read sequencing (MinIon)
EURGen-2022-04	EURGen-2022-04-FASTA-sr	short read sequencing (Illumina)
EURGen-2022-04	EURGen-2022-04-FASTA-lr	long read sequencing (MinIon)

3. Outline of the EURGEN-RefLabCap EQA 2022

3.1. Receipt of test material

In September 2022, national coordinators from the EURGen-RefLabCap project, who registered their laboratory to participate in the EURGen-RefLabCap EQA 2022, have received an email detailing how to access the ScienceData platform from where the test material (FASTA files or FASTQ files) may be downloaded. For detailed information on how to download the files, please consult Appendix 2.

3.2. Procedure and analysis of test material

Participants may decide to analyse a selection of the tests, i.e. may select to proceed with analysing *E. coli* data, only, or *Klebsiella* data, only, or may select to proceed with performing only some of the components offered as part of the EQA.

The use of the 'EURGen-RefLabCap harmonized common WGS-based genome analysis methods and standard protocols for national CCRE surveillance and integrated outbreak investigations' (see https://www.eurgen-reflabcap.eu/resources/wgs-tools) is recommended, but the participants may choose to use their current WGS analytical set-up. Thus, the participants are also asked to report method-related details in relation to the analysis performed, including the bioinformatics tools and parameters used for sequence analyses and generation of results (see also 4.1).

For the analysed FASTA files or FASTQ files, participants will be requested to submit test results listed in Table 2. Please note that only resistance genes and chromosomal mutations that are mediating resistance to any of the antimicrobials included in this EQA should be submitted to the webtool. Further details of the data submission procedure are described below (see 4.1).

Analysis of the test material must be done for each sequence individually, i.e., even if, for example, 'EURGen-2022-01-FASTA-sr' and 'EURGen-2022-01-FASTA-lr' are assemblies based on the sequence of the same bacterial isolate, the outcome of the bioinformatic analysis regarding

the components mentioned in Table 2 may not be identical. This is also relevant for the FASTQ files, where the reported results must be based on the analysis of the test material (FASTQ files) for each sequence individually.

Included classes and genes for the AMR component are as presented in Table 3.

Table 2: Test results for submission

	Type of data/test results
1	Identification of the Multi Locus Sequence Type (MLST)
2	Identification of the plasmid replicon type
3	Identification of antimicrobial resistance genes*
4	Identification of chromosomal mutations mediating antimicrobial resistance*
5	Identification of predicted antimicrobial resistance phenotype

***IMPORTANT!** Please note that only resistance genes and chromosomal mutations that are mediating resistance to any of the antimicrobials included in this EQA (see Table 3) should be submitted to the webtool.

Table 3: Antimicrobials included in the current EQA

Antimicrobial Agent	Antimicrobial Class
Amikacin	Aminoglycoside
Amoxicillin-clavulanic acid	Beta-lactam
Ampicillin	Beta-lactam
Aztreonam	Beta-lactam
Cefepime	Beta-lactam
Cefotaxime	Beta-lactam
Ceftazidime	Beta-lactam
Ceftazidime-avibactam	Beta-lactam
Ciprofloxacin	Quinolone
Colistin	Polymyxin
Ertapenem	Beta-lactam
Fosfomycin	Phosphonic antibiotics
Gentamicin	Aminoglycoside
Imipenem	Beta-lactam
Meropenem	Beta-lactam
Piperacillin-tazobactam	Beta-lactam
Tigecycline	Tetracycline
Tobramycin	Aminoglycoside
Trimethoprim	Folate pathway antagonist
Sulfamethoxazole	Folate pathway antagonist

4. Results submission

Test results are submitted via the EURGen-RefLabCap EQA 2022 webtool which refers to test material codes based on FASTA files. Laboratories which are analysing and submitting results

based on analysis of FASTQ files, must record this in the webtool under 'Method'. Here, for each sequence available for analysis, the laboratory must indicate if results were based on FASTA-files, FASTQ files, or if results for the specific test material is not submitted.

4.1. Submission of information on methods for sequence analysis and test results

Details in relation to submission of information on sequence analysis methods and sequence analysis results (see also Table 2) via the webtool are described in Appendix 3. While proceeding with the analysis, participants are invited to register relevant information using the template provided in Appendix 4 (test forms).

For results related to antimicrobial resistance, note that the analysis might require collaboration between a bioinformatician and a microbiologist with knowledge within the field of antimicrobial resistance.

As for reporting of antimicrobial resistance genes (see Table 3), participants must submit the result with the best quality in terms of percent identity and coverage (in width). All detected genes that contribute to a phenotype (mediating resistance to any of the antimicrobials included in this EQA) should be included in the data submission.

4.2. Deadline for submission of results

Submission is successful after ticking off the 'final submit' in the webtool (see webtool manual, Appendix 3). Following 'final submit', the national coordinator(s) will receive an email with the submitted results as an attachment. Results must be submitted electronically **no later than 31 October 2022 at 4 pm**. Immediately after this date, the webtool will be closed for further edits and submission. Delayed submission of results will not be accepted.

5. How to submit results via the webtool

The webtool manual (Appendix 3) presents the procedure of submission of information and results in detail, and we recommend reading it carefully.

Access the webtool using this URL: https://eurgen-reflabcap-pt.dtu.dk

When submitting results, participants are kindly invited to remember to have by their side the completed test forms (Appendix 4).

Do not hesitate to contact us if you experience difficulties with the webtool.

Before finally submitting input for the EURGen-RefLabCap EQA 2022, participants are kindly asked to ensure that they have filled in all the relevant fields as **it is possible to 'finally submit' only once!** 'Final submit' blocks any further attempt of data entry.

⇨ About login to the webtool:

When first given access to login to the webtool, the participants **personal login ID and password** were sent to the national coordinator(s) by email. This is relevant for each email address provided when registering as participant in the EQA.

6. Results evaluation

6.1. Submitted analysis methods and test results obtained from the analysis of the FASTA files or FASTQ files

The evaluation will be based on the test results submitted by the submission deadline. Results submitted to the webtool are evaluated automatically (details related to the scoring are presented in Appendix 3). Data validation will be performed by the EQA-providers after which the participants will receive an email message informing them that they may log in to the webtool once again to view and print an automatically generated report evaluating their results. The webtool will allow the participants also to download a "certificate of participation" stating that the laboratory has participated in the specific EQA without indicating pass/fail.

Evaluation criteria relevant to the submitted results are presented in the webtool manual (see Appendix 3).

Details in relation to analysis method will be used as background for the evaluation of results.

6.2. Analysis and publication of results

Each participating laboratory will receive an individual summary of the obtained performance. Moreover, an overall report summarizing the results in an anonymized form (including likely explanations for discrepant results) will be published after written consultation with the participants. The report will be shared with the HaDEA, the ECDC and will be publicly available on the EURGen-RefLabCap website.

Authors and co-authors of the publications will be those who have contributed to the preparation and execution of the EQA. Due to the anonymity of performance results, the individual participating coordinators, and colleagues in the laboratories will not be acknowledged in the publications. Instead, the participating laboratories will be asked if they would like to be acknowledged in the publications, and by which specific laboratory name, place, and organization.

Individual results will be anonymized using laboratory codes which are confidential and known only to the individual laboratory, the EQA-organizers, HaDEA, and ECDC (as advisor).

We are looking forward to receiving your results.

If you have any questions or concerns, please do not hesitate to contact us.

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PROTOCOL for EURGen-RefLabCap PT, 2022 – APPENDICES

Appendix 1 Methods used to generate the EURGen-RefLabCap EQA 2022 test sequence data

Appendix 2 Using ScienceData to transfer files

Appendix 3 EURGen-RefLabCap PT 2022 webtool manual

Appendix 4 Testforms – overview of method information to be submitted via the webtool

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Appendix 1

Methods used to generate the EURGen-RefLabCap EQA 2022 test sequence data

Test sequence code	EURGen-2022-01-sr	EURGen-2022-01-lr	EURGen-2022-02-sr	EURGen-2022-02-lr	EURGen-2022-03-sr	EURGen-2022-03-lr	EURGen-2022-04-sr	EURGen-2022-04-lr
DNA extraction kit	MagnaPure 96 DNA and Viral NA	GenFind V3	Qiagen DNeasy Blood & Tissue Kit	MagnaPure	MagnaPure 96 DNA and Viral NA	MagnaPure	MagnaPure 96 DNA and Viral NA	GenFind V3
DNA extraction protocol	MagnaPure DNA Blood ds SV	GenFind V3	*In house* modified protocol based on DNeasy® Blood & Tissue Handbook	MagnaPure	MagnaPure DNA Blood ds SV	MagnaPure	MagnaPure DNA Blood ds SV	GenFind V3
Library preparation kit	Nextera XT DNA Sample Prep Kit	SQK-RBK004	Nextera XT library Preparation Kit	SQK-RBK110.96	Nextera XT library Preparation Kit	SQK-RBK110.96	Nextera XT library Preparation Kit	SQK-RBK004
Library preparation protocol	Illumina DNA library preparation kit protocol (adapted for Hamilton Microlab Star)	SQK-RBK004	Illumina DNA library preparation kit protocol	SQK-RBK110.96	Illumina DNA library preparation kit protocol (adapted for Hamilton Microlab Star)	SQK-RBK110.96	Illumina DNA library preparation kit protocol (adapted for Hamilton Microlab Star)	SQK-RBK004
How QC of sequence data was performed	Bifrost pipeline (https://github.com/ssi-dk/bifrost) accessing genome size (1X, 10X), average coverage, species ID and unclassified reads	Quality score > 10	Bifrost pipeline (https://github.com/ssi-dk/bifrost) accessing genome size (1X, 10X), average coverage, species ID and unclassified reads	Quality score > 10	Bifrost pipeline (https://github.com/ssi-dk/bifrost) accessing genome size (1X, 10X), average coverage, species ID and unclassified reads	Quality score > 10	Bifrost pipeline (https://github.com/ssi-dk/bifrost) accessing genome size (1X, 10X), average coverage, species ID and unclassified reads	Quality score > 10
How assembly of sequence data was performed	Skesa v. 2.2	Unicycler - nanopore only	Skesa v. 2.2	Unicycler - nanopore only	Skesa v. 2.2	Unicycler - nanopore only	Skesa v. 2.2	Unicycler - nanopore only

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Appendix 2

Using ScienceData to transfer files

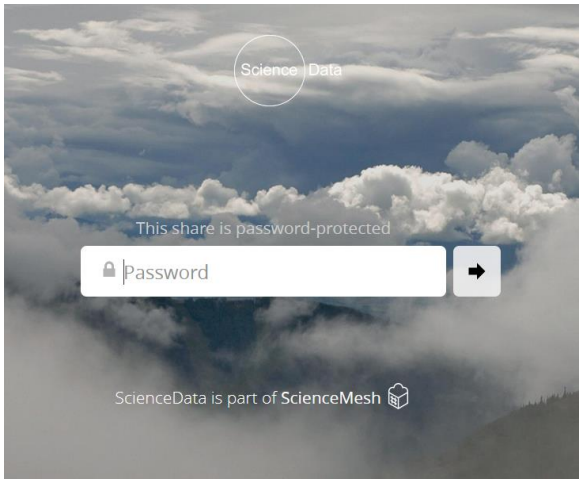
ScienceData is a cloud-based storage data platform built and operated by the Technical University of Denmark (DTU).

Obtain access to the platform by using the link and login code provided by email to contact persons when the EQA is launched.

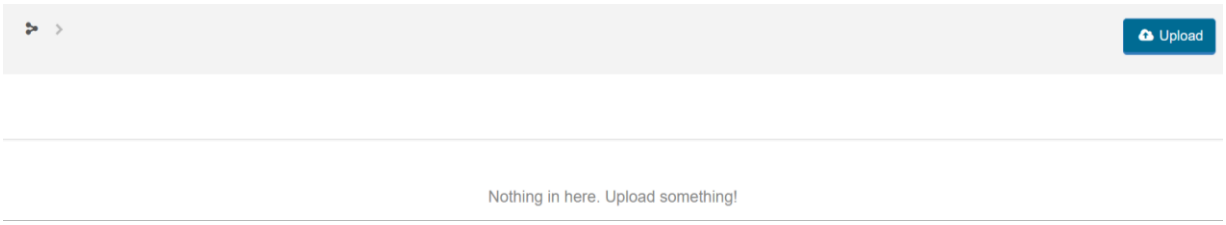
For accessing ScienceData, please use **Google Chrome**.

<u>Downloading files to the ScienceData platform</u>:

1. Click on the link provided by the EQA organizer and find the following image:

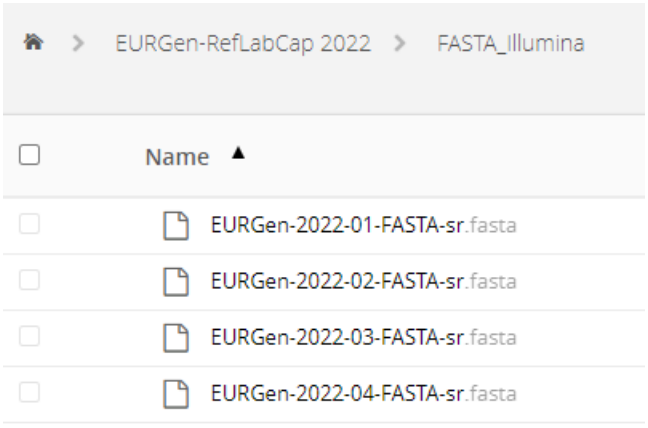


2. Type in the password provided by the EQA organizer and see following image

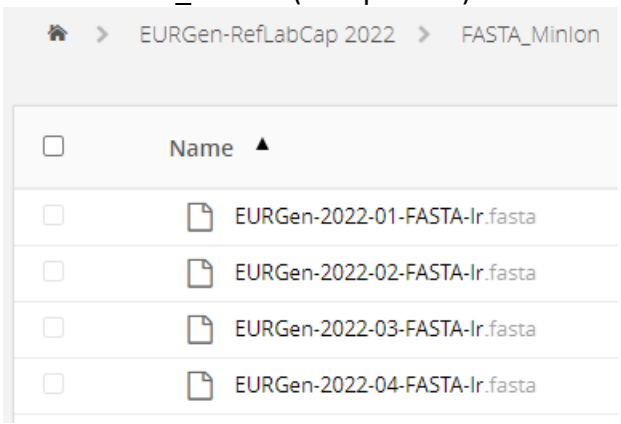


3. Click on the folder choose the files you wish to download. You should see the following sequences:

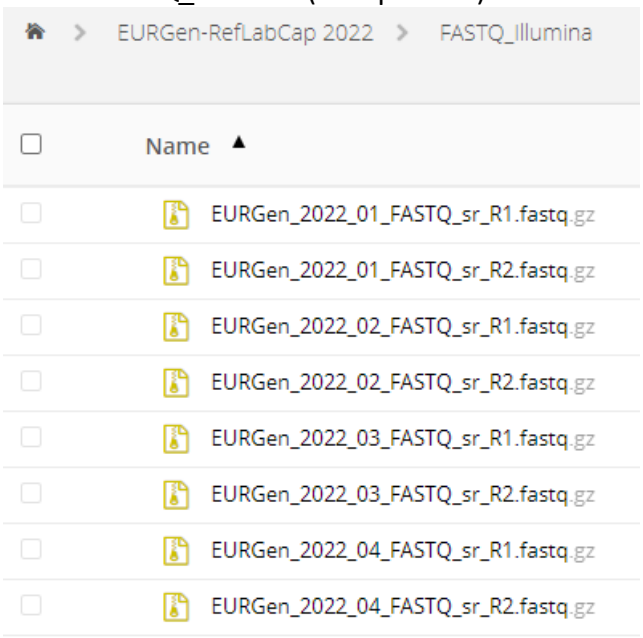
a) Folder FASTA_Illumina (4 sequences)



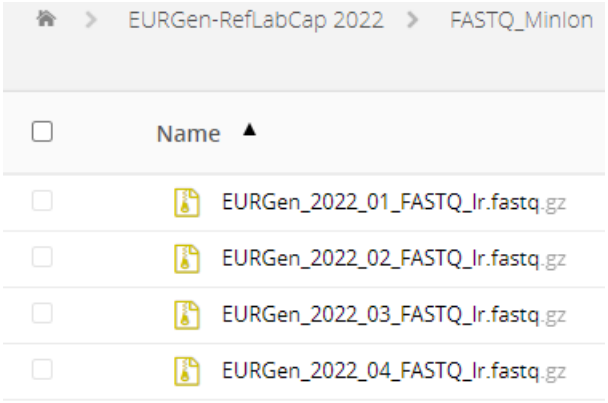
b) Folder FASTA_MinIon (4 sequences)



c) Folder FASTQ_Illumina (8 sequences)



d) Folder FASTQ_MinIon (4 sequences)



4. Mouseover the download icon to download the file of choice.

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Appendix 3

EURGen-RefLabCap EQA 2022 webtool guideline

⇨ Browser requirements

IMPORTANT: The system works with the following browsers

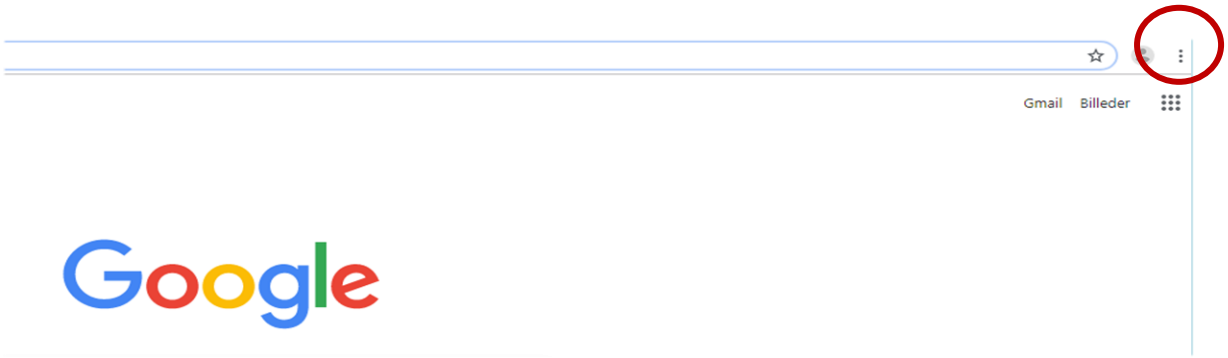
Browser	Oldest supported version*
Google Chrome	44.0
Firefox	39.0

* latest version is recommended

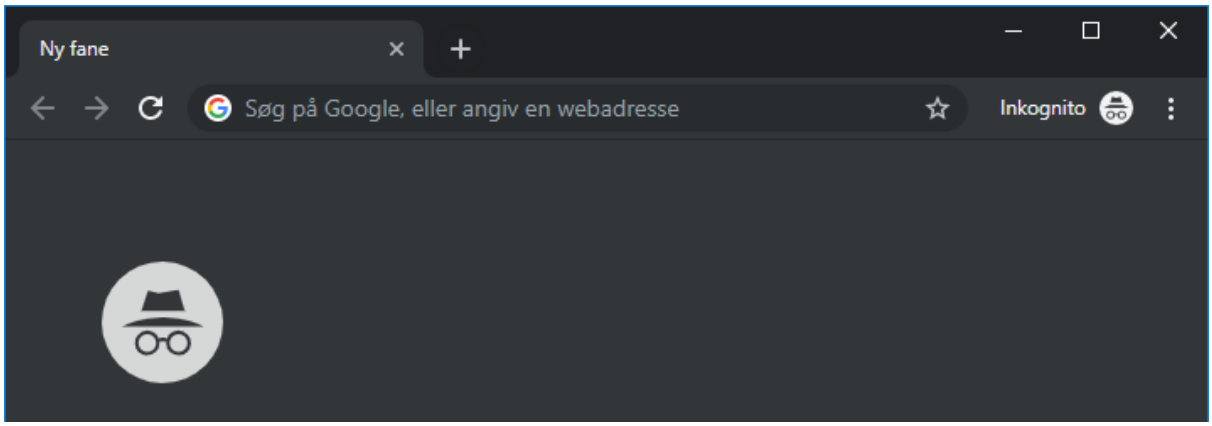
⇨ Access the webtool

IMPORTANT: To access the webtool, you must use an incognito window.
NOTE: Should you have issues with requesting an incognito window, please contact the EQA Coordinator (suska@food.dtu.dk) directly.

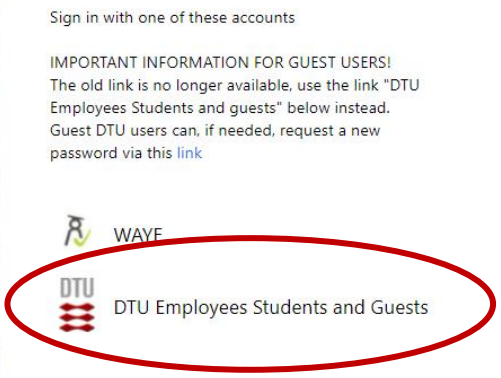
Open a browser window, click on the three dots (see red circle below) and select: 'New incognito window' (relevant when using Google chrome).



Continue in the black window that looks like this (relevant when using Google chrome):



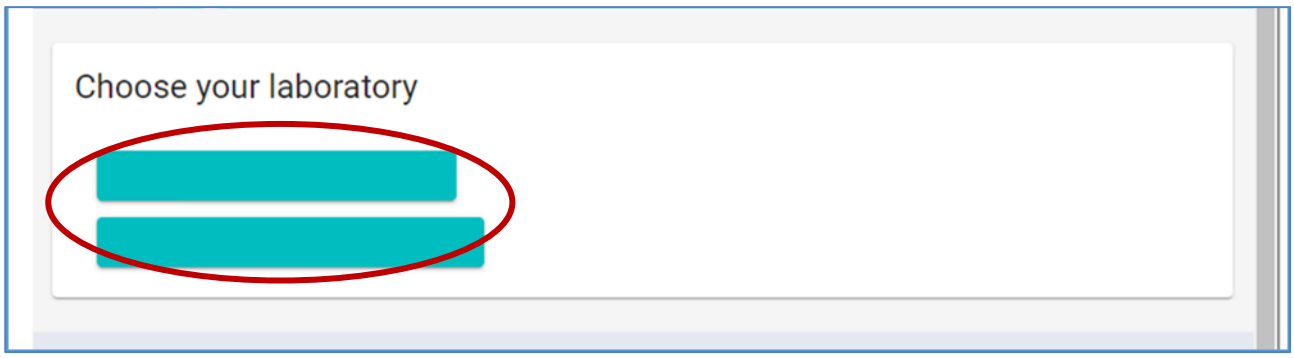
Access the webtool using this address: https://eurgen-reflabcap-pt.dtu.dk



Select: DTU Employees Students and Guests.

Login to the system by using the username and password sent to you by e-mail for participation in EQAs arranged by DTU Food.

After sign in you will reach the *Overview* page

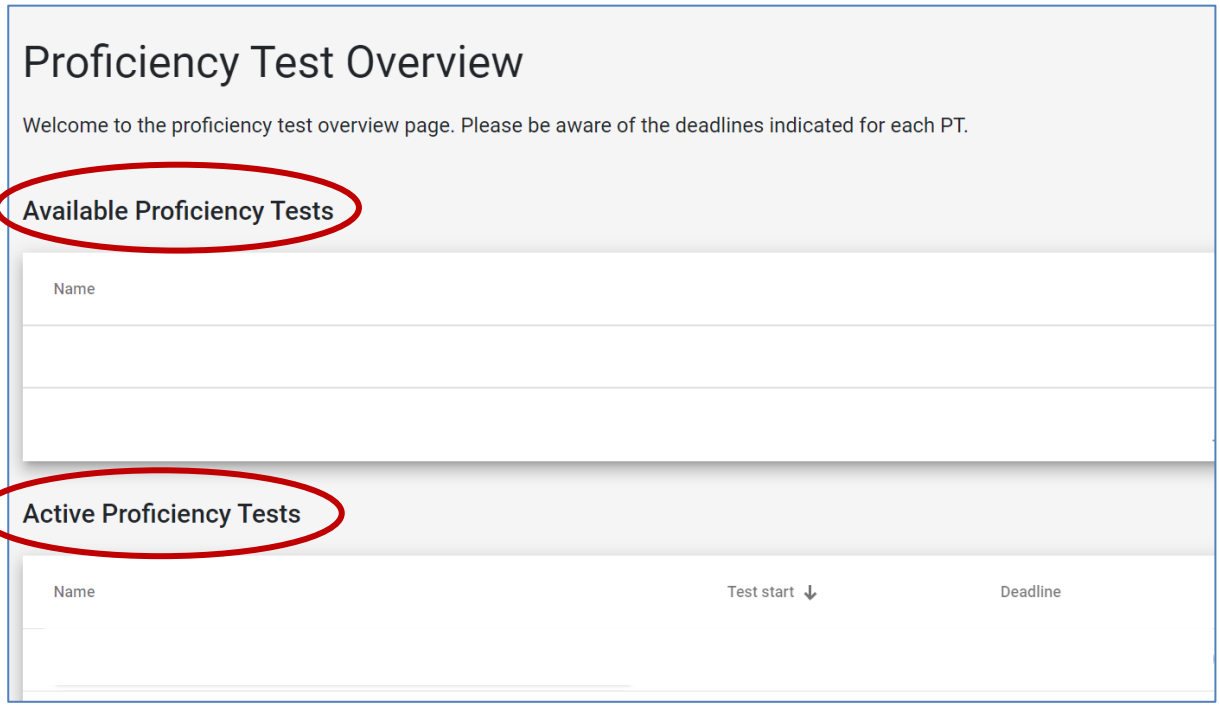


If you are connected to more than one specific laboratory, you will need to select the specific laboratory that you intend to submit results for.

If the window has been inactive for 20 minutes, the webtool will automatically time-out and present 'Access denied'. Access the webtool once again by following the above-described login procedure.

⇨ Signup or deselect

Under 'Available proficiency tests', sign-up to the relevant EQA. EQAs you signed up to will be listed under 'Active proficiency tests'. Note: for the current webtool, please read 'EQA' when reference to 'proficiency test' is made.



⇨ Navigate in the webtool

When reporting results/data in the webtool, various tabs are available:

1. **'About' tab**. With a checkmark, select the test material for which you wish to submit results, i.e. select each relevant codes
2. **'Method' tab**. Enter data regarding the sequence analysis performed
3. **'AMR' tab**. Enter data regarding antimicrobial resistance genes, chromosomal mutations mediating antimicrobial resistance and predicted phenotypic antimicrobial resistance profile
4. **'MLST' tab**. Enter data regarding sequence type and alleles
5. **'Replicon' tab**. Enter data regarding plasmid replicon type

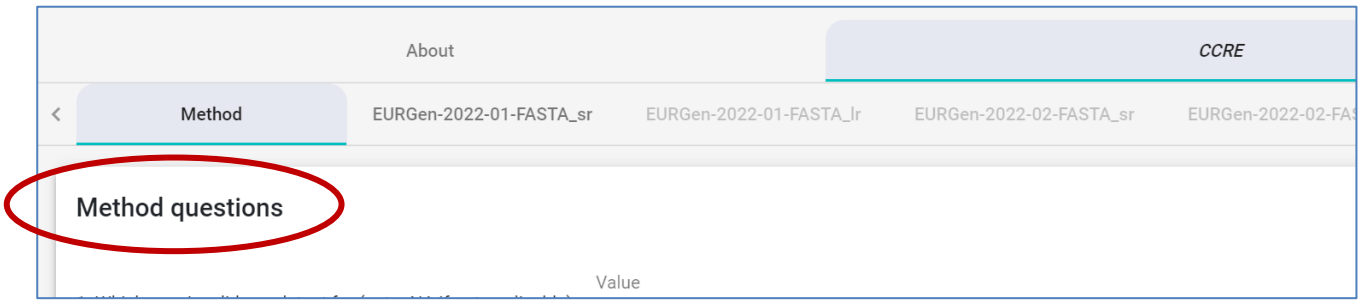
⇨ Enter data

Ad 1. 'About' tab

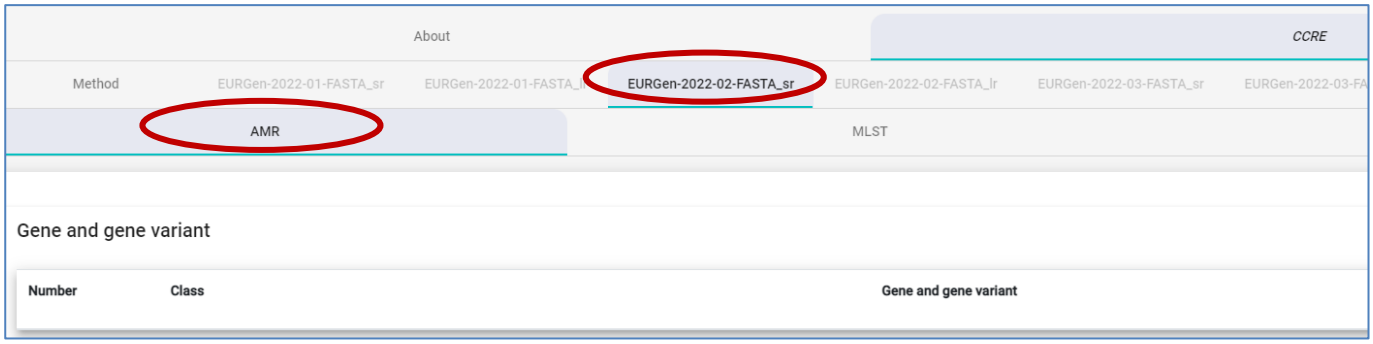
Indicate for which test material results will be submitted for. If a test material is selected under the 'About' tab, remaining tabs related to this test material will be activated and allows for access to enter results. If you are submitting results based on analysis of FASTQ files, please make checkmarks for the relevant codes even if the tab-name indicates 'FASTA' and not 'FASTQ'.

Ad 2. 'Method' tab

Respond to the questions in the 'Method' tabs.

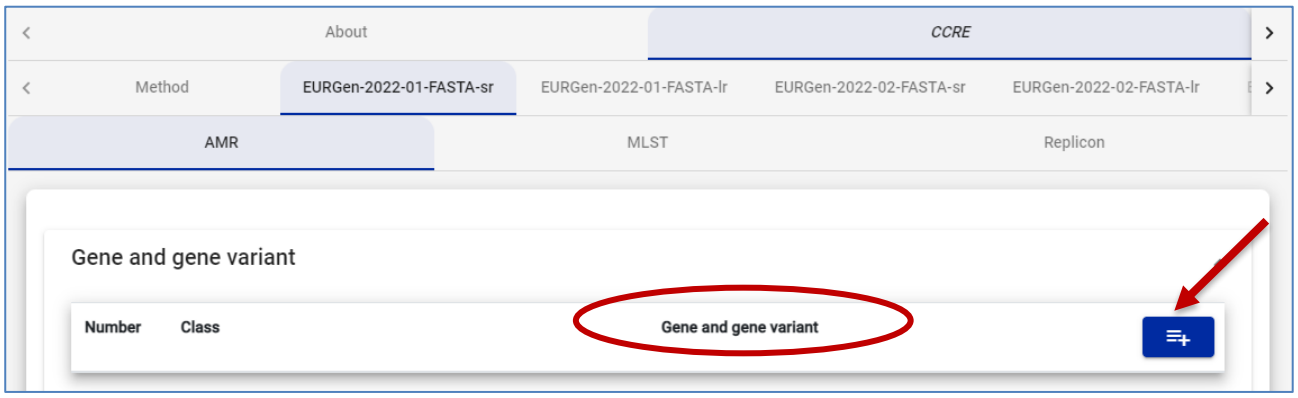


Select the tab related to one of the test strains (e.g. EURGen-2022-02-FASTA_sr). This opens for access to additional tabs. If you are submitting results based on analysis of FASTQ files, please select the tab that has the relevant code even if the tab-name indicates 'FASTA' and not 'FASTQ'.



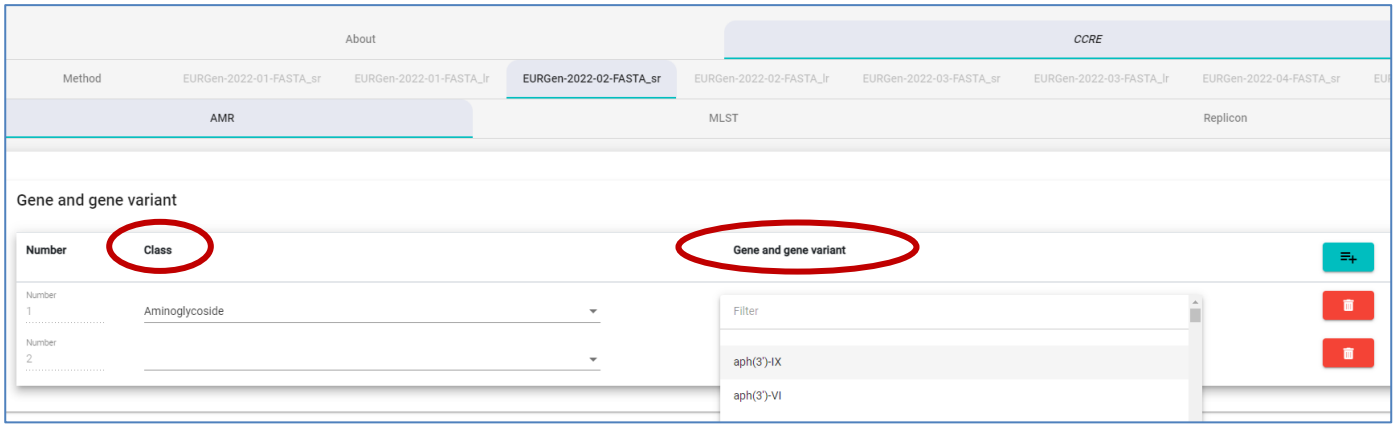
<u>Ad 3. 'AMR' tab</u>:
Under the AMR-tab, results related to 1) identified antimicrobial resistance genes, 2) identified chromosomal mutations mediating antimicrobial resistance, 3) and identified prediction of the phenotypic antimicrobial resistance profile are uploaded.

Gene and gene variant: To report genes and gene variants detected in the sequences of the test strains, please click on the '+' (see arrow below) to access the dropdown lists.



In the dropdown menu under 'Class', select the antimicrobial class of the gene and gene variant you wish to report. Hereafter, from the dropdown menu under 'Gene and gene variant', select the specific variant of the antimicrobial resistance gene you wish to report, or, to narrow down the options in the list, type (parts of) the gene variant name in the 'Filter'-field.

Add more lines by clicking on the '+' once again to proceed with submitting further genes and gene variants.



Note #1: Antimicrobial resistance towards a limited number of antimicrobial classes are considered in this EQA. Antimicrobial classes represented in the EURGen-RefLabCap EQA 2022, and consequently in the webtool drop down list are:

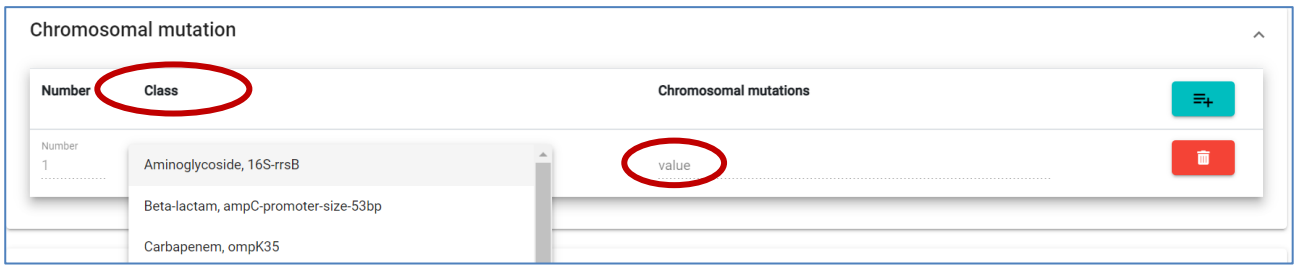
Included classes and genes for the AMR component are as presented in Table 3 in the protocol text. All genes conferring resistance to the antimicrobials in this list should be reported, *i.e.* including genes associated with intrinsic and acquired resistance.

Some antimicrobial resistance genes are associated with resistance to more than one class of antimicrobials, to select these genes, select a 'Class' containing multiple classes and subsequently select the specific gene and gene variant.

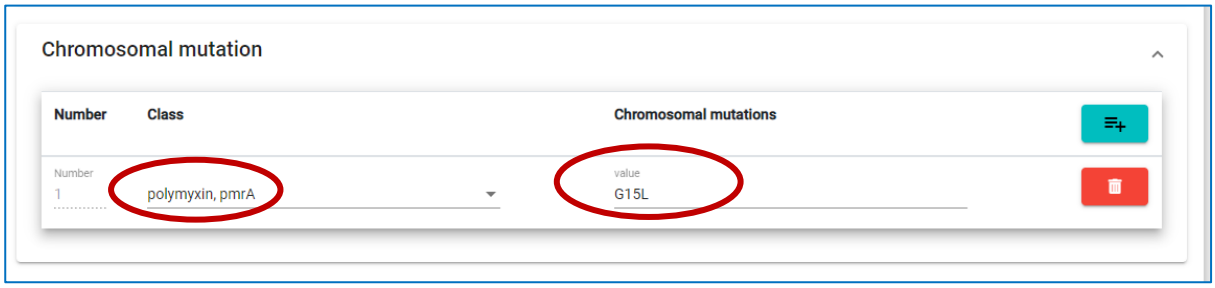
Ensure that no empty lines are saved for evaluation by clicking on the bin if you by mistake added one too many.

'Chromosomal mutations': In the dropdown menu under 'Class', select the specific class of antimicrobial followed by the mutated gene you wish to report a chromosomal mutation for. Hereafter, under **'**Chromosomal mutations', in the empty field named "value", write the specific mutation as follows:

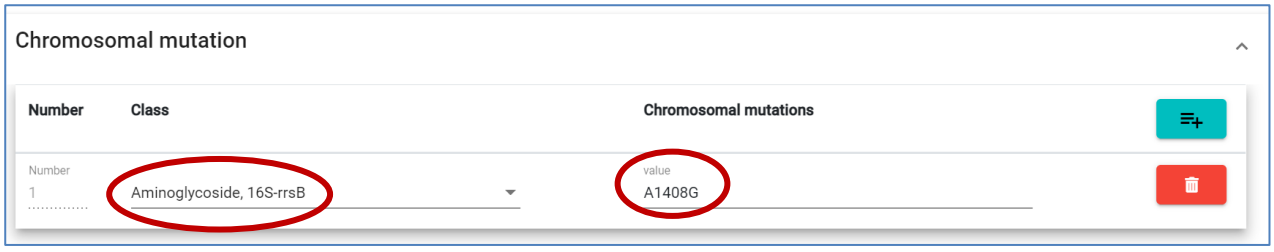
1) Indicate the reference codon (an amino acid letter, or a nucleotide letter for 16S or 23S sequences)
2) Indicate the position of the codon (a numeric value)
3) Indicate the resistance codon (amino acid letter, or nucleotide for 16S or 23S sequences)



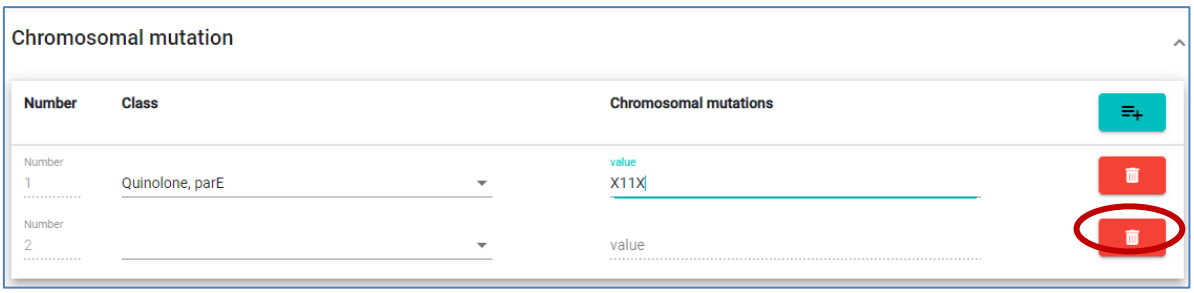
Example 1: Reporting a mutation in the *pmr*A gene which has changed the *amino acid* glycine (G) to Leucine (L) at position 15. This results in resistance to colistin that belongs to the polymyxin class of antimicrobials. Therefore, from the dropdown list under 'Class', select the 'polymyxin, *pmr*A' option and write G15L in the 'value' field under 'Chromosomal mutations'.



Example 2: If the mutation is in a 16S rRNA gene please select the class of antimicrobial and associate gene (e.g. Aminoglycoside, 16S-rrsB) from the dropdown menu. Hereafter, in the 'value', write the letter of the original reference *nucleotide* (A, T, C or G) and its position, followed by the new nucleotide letter that the mutation has resulted in (e.g. A1408G). Same principle as for the amino acids.

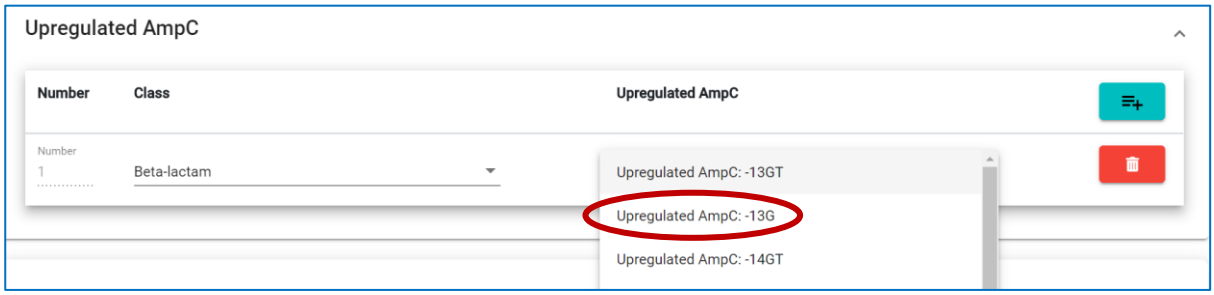


Ensure that no empty lines are saved for evaluation by clicking on the bin if you by mistake added one too many.

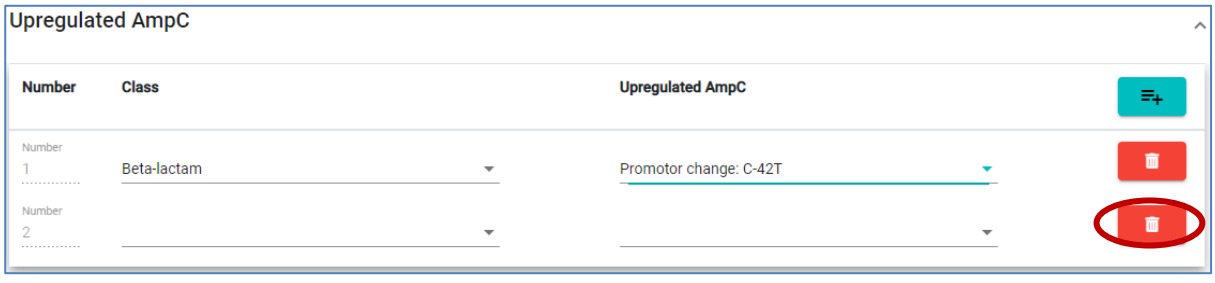


'Upregulated ampC': Upregulated ampC resistance can be reported by selecting the 'Upregulated ampC' option under the 'AMR' tab. For the 'Upregulated ampC' option, select 'Beta-lactam' under 'Class' and hereafter, from the dropdown menu under 'Upregulated ampC', select the specific mutations in the promoter region.

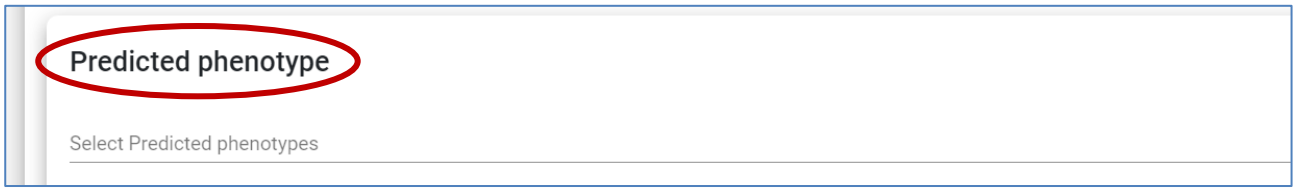
The mutations are shown in the same way as previously described for 16S and 23S sequence mutations, i.e. the reference codon is followed by a numeric value, and then followed by the resistance codon (unless the mutation is an insertion). Since the promoter is located upstream to the open reading frame (ORF) a minus (-) is found before the position number. e.g. C-42T (indicating that the nucleotide cytosine (C) has been exchanged with thymine (T)). Regarding insertions, there is no reference nucleotide, therefore, for example, the indication '-13G' represents the nucleotide guanine (G) inserted at position -13 (upstream the ORF).



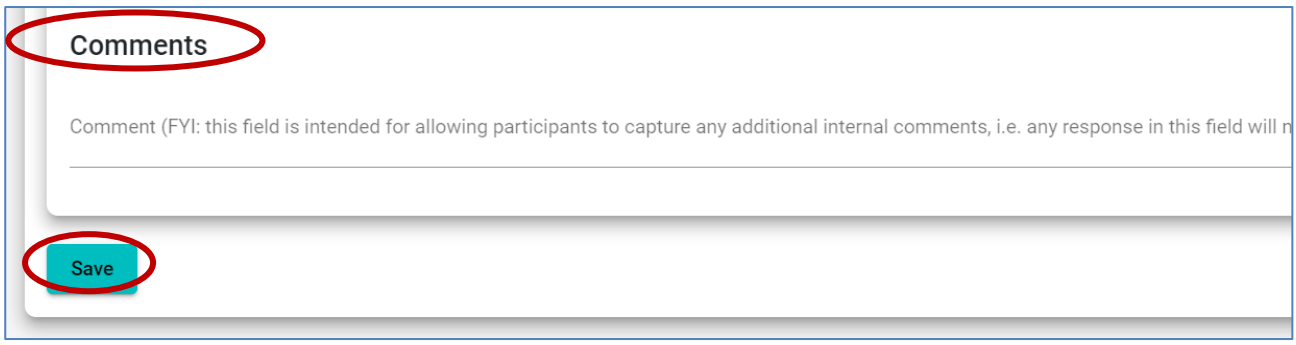
Ensure that no empty lines are saved for evaluation by clicking on the bin if you by mistake added one too many.



'Predicted phenotype': Click on 'Predicted phenotype' to select the antimicrobials that the sequence analysis indicates resistance to in the bacterial test strain. Note that in relation to predicted phenotype, antimicrobial resistance towards a limited number of antimicrobials are considered in this EQA. Antimicrobials represented in the EURGen-RefLabCap EQA are presented in Table 3 if the protocol text.

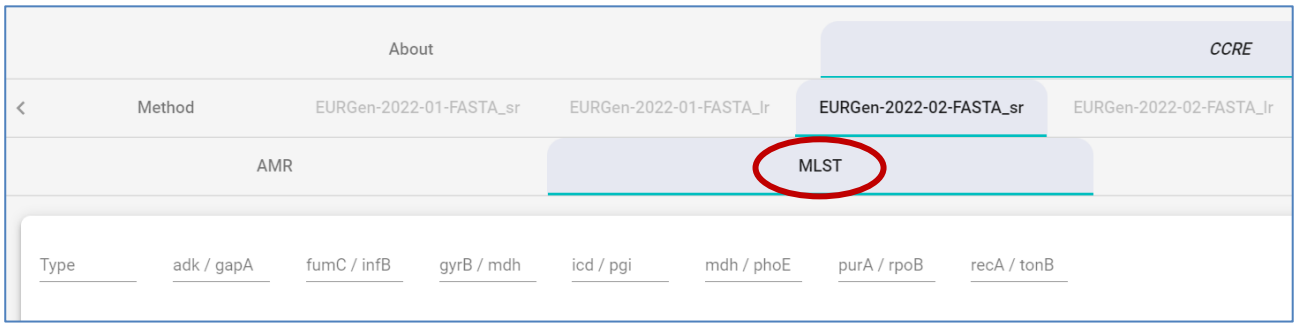


'Comment': Any comments related to the submission of the results are welcome. You may for example indicate mutations that have unknown effect on antimicrobial resistance. You may also report genes and/or mutations that were not listed in the drop-down menu of the tabs described above. Note, however, that these results will *not* be further evaluated.



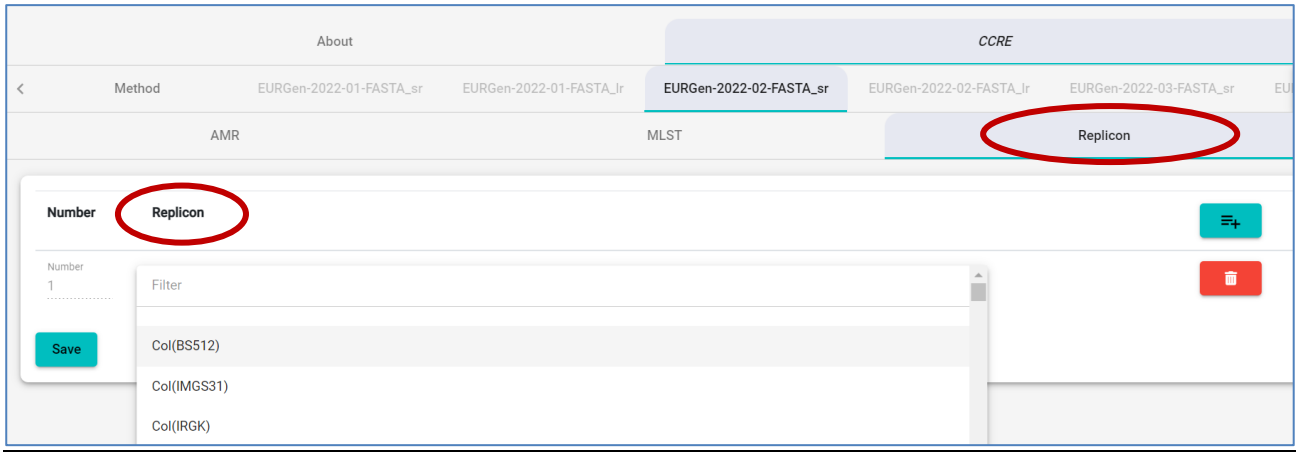
Ad 4. 'MLST' tab

Enter data regarding MLST results (MLST type and corresponding allele numbers). Enter "0" if the obtained result does not show a perfect match or if an allele cannot be detected.



Ad 5. 'Replicon' tab

Enter data regarding plasmid replicon type by selecting from the dropdown list.



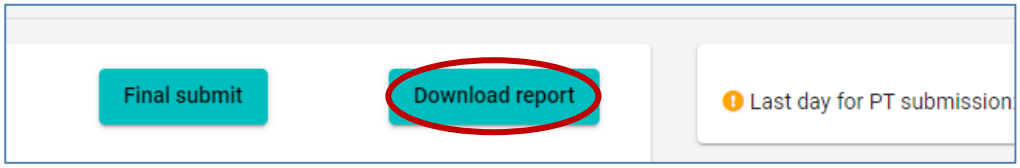
⇨ Save data

Data are saved when you click the *save* button on each page.

Moreover, data are saved when you navigate to another tab.

⇨ Review and revise data

On the *Proficiency Test Overview* page as well as in the *Test overview page*, click 'Download report' to see the overview of your results and method input for this EQA.



Before you have finally submitted your results (and before deadline), the database allows you to return to any test form and revise values.

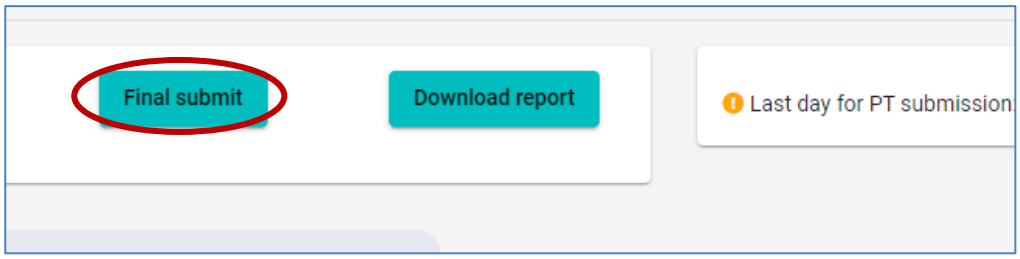
⇨ Submit data

For all test material of the EURGen-RefLabCap EQA 2022, all uploaded data are submitted in one go.

When all information and data have been entered and revised for i) the method, and, ii) FASTA files (or FASTQ files), please indicate with a checkmark your acceptance that the uploaded data are ready for submission.

IMPORTANT! You will **NOT** be able to edit your data after final submission.

Click on 'Final Submit'



When you have finally submitted, the *Proficiency Test Overview* page will indicate the submission status of your Proficiency Test to be 'Yes'

⇨ Evaluation and score

Submitted results are evaluated according to the following details:

Reported antimicrobial resistance genes, for a given test strain genome, will be compared to the expected results for the list of included antimicrobials. When a submitted antimicrobial resistance gene is on the list of expected antimicrobial resistance genes (i.e. if the obtained and expected antimicrobial resistance genes match), a score of '1' will be obtained whereas a mismatch (obtained result is not expected) is scored with '0'. Expected antimicrobial genes which are not submitted as obtained results will be listed in the evaluation report and for these the score field(s) will be blank.

The reported chromosomal mutations mediating antimicrobial resistance for a given test strain genome, will be compared to the expected results for the list of included antimicrobials. When a submitted chromosomal mutation mediating antimicrobial resistance is on the list of expected chromosomal mutations mediating antimicrobial resistance (i.e. if the obtained and expected results match), a score of '1' will be obtained whereas a mismatch (obtained result is not expected) is scored with '0'. Expected chromosomal mutations mediating antimicrobial resistance which are not submitted as obtained results will be presented in the evaluation report and for these the score field will be blank.

Reported predicted phenotypic antimicrobial resistance profile for a given test strain genome, will be compared to the expected results for the list of included antimicrobials. If the obtained and expected results match, a score of '1' will be obtained whereas a mismatch is scored with '0'. Individual evaluation of each antimicrobial will not be performed.

The reported Multi Locus Sequence Type (MLST) will be compared to the MLST of the reference sequence. A match of the obtained and expected MLST is scored with '1'. A mismatch is scored with '0'.

Similarly, reported plasmid replicon type will be compared to the expected plasmid replicon type. A match of the obtained and expected replicon type is scored with '1'. A mismatch is scored with '0'.

When the score is released and the evaluation reports are accessible, all participating laboratories will receive an email message from the EQA organizer. Upon login to the database, clicking on '*Download report*' will give access to the report presenting obtained results, expected results and scores.

The evaluation will not indicate pass/fail.

⇨ Support

Should you need support in using the webtool, please do not hesitate to contact the EQA Coordinator (suska@food.dtu.dk).

See also the top right corner of all pages in the webtool to find the name and email address for the EQA organizer. Find also link to the EURGen-RefLabCap website (to access the relevant EQA protocol) as well as access to the current webtool manual.

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Appendix 4

Testforms – overview of method information to be submitted via the webtool

With this document, we present an overview of the data that must/may be submitted to the webtool.

You will find questions in relation to the methods for analysis of the received sequences (FASTA-files or FASTQ-files). The responses to these questions will used for the analysis of the submitted results and to benchmark tools and databases for typing of bacteria (MLST and plasmid replicon typing) and for detection of antimicrobial resistance genes.

Please note that the EURGen-RefLabCap EQA 2022 webtool refers to test material codes based on FASTA files. Laboratories which are analysing and submitting results based on analysis of FASTQ files, should record this under 'Method'. Here, for each sequence available for analysis, the laboratory must indicate if results were based on FASTA-files, FASTQ files, or if results for the specific test material is not submitted.

Note: An asterisk (*) indicates a question that requires an answer.

1. For EURGen-2022-01 (short read sequences), which type of sequences did you analyse and submit data for:
    a) FASTA
    b) FASTQ
    c) No results submitted

2. For EURGen-2022-01 (long read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

3. For EURGen-2022-02 (short read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

4. For EURGen-2022-02 (long read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

5. For EURGen-2022-03 (short read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

6. For EURGen-2022-03 (long read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

7. For EURGen-2022-04 (short read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

8. For EURGen-2022-04 (long read sequences), which type of sequences did you analyse and submit data for:
    a. FASTA
    b. FASTQ
    c. No results submitted

9. Which species did you detect for EURGen-2022-01-FASTA
    a. E. coli
    b. Klebsiella pneumoniae
    c. No results submitted

10. Which species did you detect for EURGen-2022-02-FASTA
    a. E. coli
    b. Klebsiella pneumoniae
    c. No results submitted

11. Which species did you detect for EURGen-2022-03-FASTA
    a. E. coli
    b. Klebsiella pneumoniae
    c. No results submitted

12. Which species did you detect for EURGen-2022-04-FASTA
    a. E. coli
    b. Klebsiella pneumoniae
    c. No results submitted

13. For the detection of Multi Locus Sequence Type, which methods did you apply?* (Enter 'NA' if not applicable)
    Please report information regarding:
    a. Pipeline type: local or web-based pipeline
    b. Software: publicly available software, commercial software, or in-house scripts. If you used a software, please report the name and the version number. If you used an in-house script, please specify the program
    c. Database: publicly available database, commercial database, or an in-house database. If you used an available database, please report database name and version number. If you used an in-house database, please specify the loci included in the scheme
    d. Parameters of the software: default parameters or defined by you. If you used parameters defined by you, please specify them
    e. If applicable, please specify the URL of the software and/or database used

14. For the detection of plasmids, which detection and/or typing methods did you apply?* (Enter 'NA' if not applicable)
    Please report information regarding:
    a. Pipeline type: local or web-based pipeline
    b. Software: publicly available software, commercial software, or in-house scripts. If you used a software, please report the name and the version number. If you used an in-house script, please specify the program
    c. Database: publicly available database, commercial database, or an in-house database. If you used an available database, please report database name and version number. If you used an in-house database, please briefly describe the sequences included in the database
    d. Parameters of the software: default parameters or defined by you. If you used parameters defined by you, please specify them (*e.g.* minimum length 80% and minimum identity 95%, etc.)
    e. If applicable, please specify the URL of the software and/or database used

15. For the detection of antimicrobial resistance genes, which methods did you use?* (Enter 'NA' if not applicable)
    Please report information regarding:
    a. Pipeline type: local or web-based pipeline
    b. Software: publicly available software, commercial software, or in-house scripts. If you used a software, please report the name and the version number. If you used an in-house script, please specify the program
    c. Database: publicly available database, commercial database, or an in-house database. If you used an available database, please report database name and version number. If you used an in-house database, please briefly describe the genes included in the database
    d. Parameters of the software: default parameters or defined by you. If you used parameters defined by you, please specify them (*e.g.* minimum length 80% and minimum identity 95%, etc.)
    e. if applicable, please specify the URL of the software and/or database used

16. For the detection of chromosomal mutations mediating antimicrobial resistance, which methods did you apply?* (Enter 'NA' if not applicable)
    Please report information regarding:
    a. Pipeline type: local or web-based pipeline
    b. Software: publicly available software, commercial software, or in-house scripts. If you used a software, please report the name and the version number. If you used an in-house script, please specify the program
    c. Database: publicly available database, commercial database, or an in-house database. If you used an available database, please report database name and version number. If you used an in-house database, please briefly describe the point mutations included in the database
    d. Parameters of the software: default parameters or defined by you. If you used parameters defined by you, please specify them (*e.g.* minimum length 80% and minimum identity 95%, etc.)
    e. If applicable, please specify the URL of the software and/or database used

17. For the WGS-based prediction of antimicrobial resistance phenotypes, which methods did you apply?* (Enter 'NA' if not applicable)
    Please report information regarding:
    a. Pipeline type: local or web-based pipeline
    b. Software: publicly available software, commercial software, or in-house scripts. If you used a software, please report the name and the version number. If you used an in-house script, please specify the program
    c. Database: publicly available database, commercial database, or an in-house database. If you used an available database, please report database name and version number. If you used an in-house database, please briefly describe the sequences included in the database
    d. Parameters of the software: default parameters or defined by you. If you used parameters defined by you, please specify them (*e.g.* resistance is called if gene is present with minimum length 100% and minimum identity 98%, etc.)
    e. If applicable, please specify the URL of the software and/or database used

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