



PROTOCOL for DTU Genomic Proficiency Test 2020

1	OVERVIEW AND OBJECTIVES.....	2
2	INTRODUCTION	2
3	OUTLINE OF THE DTU GENOMIC PT 2020	3
3.1	Shipping, receipt and storage of bacterial strains.....	3
3.2	Supplied test material	4
3.3	Procedure and analysis of test material	5
4	RESULTS SUBMISSION	5
4.1	Transferring FASTQ-files using FTP.....	5
4.2	Submission of sequence files	5
4.3	Submission of method information and results from the sequence analysis.....	6
4.4	Deadline for submission of results.....	6
5	HOW TO SUBMIT RESULTS VIA THE WEBTOOL	6
6	RESULTS EVALUATION	7
6.1	Submitted sequences	7
6.2	Submitted method details and results from the analysis of the obtained sequences	8
6.3	Analysis and publication of results.....	8

APPENDICES

HISTORY OF CHANGES:

Version 2 includes Appendix 2 'DTU Genomic PT webtool guideline'



1 OVERVIEW AND OBJECTIVES

The DTU Genomic proficiency test (PT) 2020, consists of two general parts:

- 1a. DNA extraction, purification, library-preparation, and whole genome sequencing (WGS) from **live cultures**
- 1b. WGS of **pre-prepared DNA**

NB: Please pay careful attention to instructions regarding the format and naming of results files submitted to the ftp site

The main **objective** of this proficiency test is to quantify differences among laboratories in order to facilitate the development of reliable laboratory results of consistently good quality within the area of DNA preparation, sequencing, and analysis (e.g. detection of antimicrobial resistance (AMR) genes, MLST, serotype). This ensures that the discrepancies and differences among laboratories are known and will contribute to the standardization of WGS and data analysis, with the aim to produce comparable data for the monitoring and research purposes.

The DTU Genomic PT 2020 is coordinated by the National Food Institute, Denmark and funded by the Fleming Fund (SEQAFRICA Regional Grant) and the EU Commission (via the EU Reference Laboratory for Antimicrobial Resistance). The Fleming Fund is a £265 million UK aid investment to tackle antimicrobial resistance in low- and middle-income countries around the world. The programme is managed by the UK Department of Health and Social Care, in partnership with Mott MacDonald, the Fleming Fund Grants Management Agent). In addition, the DTU Genomic PT 2020 is supported by WHO (via the WHO Collaborating Centre for antimicrobial resistance and genomics), FAO (via the FAO Reference Laboratory for antimicrobial resistance), and the US Food and Drug Administration (closing of reference genomes).

2 INTRODUCTION

The proficiency test programme focuses on *Salmonella enterica*, *Escherichia coli*, *Campylobacter coli*/*Campylobacter jejuni* and allows for sign-up for each organism, separately. Note that item 1a and item 1b are parallel; i.e. when signing up for 1a for one organism, the participation in 1b is also expected.

The two items consist of

- 1a) DNA extraction, purification, library-preparation, and WGS of six bacterial cultures: two *Salmonella enterica* strains, two *E. coli* strains and two *Campylobacter* strains.
- 1b) WGS of pre-prepared DNA of the same six bacterial strains mentioned in clause 1a.



Institutes/organizations that signed up to participate will receive the PT-material (cultures/pre-prepared DNA) according to the registered sign-up information.

For the test strains that are analysed, participants will be requested to submit the data and test results for evaluation listed in Table 1.

Table 1: Data/test results requested for submission

Type of data/test results		
1	Upload reads of the sequenced culture/pre-prepared DNA to an ftp-site (FASTQ-files)	Mandatory
2	Details in relation to received test material	Mandatory
3	Details in relation to sequencing and analysis method	Mandatory
4	Identification of the Multi Locus Sequence Type (MLST) of the sequenced culture/pre-prepared DNA	Mandatory
5	Identification of antimicrobial resistance genes, chromosomal mutations inducing antimicrobial resistance, upregulated AmpC (relevant for <i>E. coli</i>) and subsequently identification of the predicted phenotype of the culture/pre-prepared DNA).	Mandatory for laboratories part of the SEQAFRICA grant and laboratories belonging to the EURL-AR network Optional for all other participating laboratories
6	Identification of serogroup/serotype (relevant for <i>Salmonella</i> and <i>E. coli</i>)	Optional

3 OUTLINE OF THE DTU GENOMIC PT 2020

3.1 Shipping, receipt and storage of bacterial strains

In September 2020, laboratories located worldwide will receive a parcel containing two strains of *Salmonella enterica*, two strains of *Escherichia coli*, as well as two *Campylobacter* strains together with corresponding pre-prepared DNA (contents of the parcel will correspond to the registered sign-up information). All bacterial strains and DNA are shipped as UN3373, Biological substance category B.

Please confirm receipt of the parcel through the confirmation form enclosed in the shipment

The *Salmonella* and *E. coli* strains are shipped as agar stab cultures whereas the *Campylobacter* strains are shipped as charcoal swab in a transport media. **On arrival**, the stab cultures and the charcoal swabs must be subcultured, and all cultures should be adequately stored until handling in the laboratory.



The pre-prepared DNA is shipped as dried samples using a DNA stabilizing agent (DNAstable® Plus, Biomatrixa, <https://www.biomatrixa.com/download/hp-dnastable-plus-handbook/>). **On arrival**, either rehydrate your sample (please see 3.2.2 Item 1b; DNA) and store the liquid samples at room temperature in closed tubes, to prevent evaporation. Or store the dried samples in either

- (a) A dry storage cabinet at room temperature (15-25°C or 59-77°F) or
- (b) A heat-sealed, moisture-barrier bag along with a silica gel desiccant pack.
- (c) If sequencing of the samples is planned within the first 10 days of arrival of the shipment, you may store the dried samples in the zip-lock bag in which they arrived along with the silica gel desiccant pack. If moisture starts to appear, the desiccant pack must be changed.

3.2 Supplied test material

3.2.1 Item 1a; Bacterial cultures

The bacterial cultures supplied have been sequenced multiple times and the genomes have been closed.

The PT-organizers therefore encourage participants to subculture and prepare the bacterial cultures for storage in your strain collection (e.g. in a -80°C freezer) with the purpose of future internal quality control and to serve as reference if discrepancies are detected during the testing (e.g. they can be used to detect errors such as mis-labelling or contamination).

3.2.2 Item 1b; DNA

The supplied pre-prepared DNA has been stabilized by DNA Stable® plus (<https://www.biomatrixa.com/download/hp-dnastable-plus-handbook/>). Each vial contains a minimum of 2 µg DNA.

Before use, the supplied DNA should be rehydrated. Add 60 – 100 µl nuclease free water or aqueous buffer to the dried DNA. Incubate the tubes at room temperature for 15 minutes to allow complete hydration. Gently mix the sample by pipetting, to re-suspend the sample. The rehydrated DNA can now be used directly in downstream application.

Unused rehydrated sample can be stored for up to one month at 4 degrees or room temperature, or longterm at -80°C.

Optional: The quality of the rehydrated DNA can be checked and visualized by agarose gel electrophoresis. The amount of DNA supplied in each tube is sufficient to run a small fraction on a gel.



3.3 Procedure and analysis of test material

3.3.1 Item 1a and 1b; Bacterial cultures and DNA

Subculture the bacterial strains on a growth medium relevant for the organism in question and incubate. Following incubation and assessment of purity of the bacterial cultures, perform DNA extraction and WGS according to the laboratory's standard procedure.

For the pre-prepared DNA received, perform WGS according to the laboratory's standard procedure.

While handling both bacterial cultures and pre-prepared DNA (items 1a and 1b), register relevant information. For this purpose, Appendix 3 (testforms) gives an overview of the requested data (information and results) relevant to the sequencing and the analysis of the sequences, whereas Appendix 2 (manual for the webtool) presents the information on how to access the webtool and the procedure for submission of data.

4 RESULTS SUBMISSION

For results submission, both transferring of FASTQ-files to an ftp-site and submission of data via the DTU Genomic PT webtool is relevant.

4.1 Transferring FASTQ-files using FTP

For submission of sequencing results (FASTQ-files), an ftp-server is used. The proficiency test organizer provides each participant with username and login for this purpose (see letter that accompanied your test strains/DNA). The ftp-site which will be used for this purpose is `cgebase.cbs.dtu.dk`. For information on how to transfer files, please consult Appendix 1.

4.2 Submission of sequence files

Before submission of the FASTQ-files, rename them to match corresponding samples as follows: GENOMIC20-003-BACT should be called GENOMIC20-003-BACT_R1.fastq.gz and GENOMIC20-003-BACT_R2.fastq.gz (if pair-end sequencing), and GENOMIC20-003-BACT_R1.fastq.gz (if single-end sequencing).

Transfer FASTQ-files related to item 1a and 1b to your ftp-folder. Files available in the ftp-folder by the submission deadline are considered for evaluation. Subsequently, pre-screening steps will be performed to check the sequence file format (FASTQ) and file name (as described above). If the



file format and file name are not compatible with the submission guideline, the file will be excluded from further analysis.

4.3 Submission of method information and results from the sequence analysis

Details in relation to submission of method information and results from the sequence analysis (see also Table 1) via the webtool are described in Appendix 2. Test forms that present an overview for recording your results before you enter them into the web tool are available in Appendix 3. This is relevant for:

- Details in relation to received test material (mandatory).
- Details in relation to sequencing and analysis method (mandatory).
- Identification of the Multi Locus Sequence Type (MLST) of the sequenced culture and pre-prepared DNA (mandatory).
- Identification of antimicrobial resistance genes, chromosomal mutations inducing antimicrobial resistance, upregulated AmpC (relevant for *E. coli*) and subsequently identification of the predicted phenotype of the culture/pre-prepared DNA). (Mandatory for laboratories belonging to the EURL-AR network and for laboratories part of the SEQAFRICA grant. Optional for all other participating laboratories).
- Identification of serogroup/serotype (relevant for *Salmonella* and *E. coli*) (optional).

All laboratories are invited to submit results related to antimicrobial resistance, though it is mandatory for some participating laboratories, only. If submitting results, note that the analysis related to antimicrobial resistance might require collaboration between a bioinformatician and a microbiologist with knowledge within the field of antimicrobial resistance.

4.4 Deadline for submission of results

Submission is successful after ticking off the 'final submit' in the webtool (see webtool manual, Appendix 2). Following 'final submit', the primary and secondary contact person receives an email with the submitted results as an attachment. Results must be submitted electronically **no later than 11 December 2020**. 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 2) presents the procedure of submission in detail and we recommend to read it carefully before submitting your results (method details and results from the analysis of the obtained sequences).

Access the webtool using this URL: <https://genomic-pt.dtu.dk>.



When you submit your results, remember to have by your side the completed test forms (Appendix 3).

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

Before finally submitting your input for the DTU Genomic PT 2020, please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once!** 'Final submit' blocks data entry.

⇒ About login to the webtool:

When first given access to login to the webtool, your **personal loginID and password** were sent to you by email. This is relevant for two email addresses connected to each participating laboratory. The PT organizers have defined a primary and a secondary contact based on the contact persons registered at the sign-up.

Note that:

- a) If the PT organizers has only one contact person for a participating laboratory, this person is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact suska@food.dtu.dk
- b) If your laboratory has registered two or more contact points, two have been defined as the primary and secondary contact. Should you like to make changes to the primary and secondary contact or should you like more than the two persons to be able to access the webtool, please contact suska@food.dtu.dk.

6 RESULTS EVALUATION

6.1 Submitted sequences

For both bacterial cultures and pre-prepared DNA (items 1a and 1b), the evaluation will be based on the submitted sequence data (FASTQ-files) which will be assembled using SPAdes [<http://bioinf.spbau.ru/spades>] and run through a QC pipeline by the PT-organizers. The output from the QC analysis are collected in two tables: a summarizing scoring table and an elaborating QC parameter table. The QC parameter table contains the specific values from the QC analysis, including (but not limited to) number of reads, coverage, depth of coverage, number of contigs and N50. The scoring table sums up the general performance of each sample based on the following criteria: average coverage, average insert size, MLST type, number of ribosomal gene differences, Q-score of R1, Q-score of R2 and size of the assembled genome.



When receiving the results evaluation, each participant is asked to assess their own performance and consider whether the obtained results should lead to adjustments internally, considering their handling of bacterial strains and/or DNA sequencing.

Output from the QC pipeline will be evaluated by the PT-organizers to identify underperformance, based on outlying results.

6.2 Submitted method details and results from the analysis of the obtained sequences

After the submission deadline, when all participants have uploaded results, you will be able to login to the webtool once again to view and print an automatically generated report evaluating your results. Results submitted to the webtool are evaluated automatically and upon deadline, an evaluation report may be extracted.

Evaluation criteria relevant to the submitted results are presented in the webtool manual (see Appendix 2).

Details in relation to received test material and in relation to sequencing and analysis method will be used as background for the analysis.

6.3 Analysis and publication of results

Each participating laboratory will receive an individual summary of the obtained performance, moreover, an overall report summarizing the results will be published and possibly subsequently in a peer-reviewed publication. Authors and co-authors of the publications will be those who have contributed to the preparation and execution of the proficiency test. Due to the anonymity of results, the individual participating laboratories will not be acknowledged in the publications.

Individual results will be anonymized using laboratory codes which are confidential and known only to the individual laboratory and the PT-organizers. For laboratories participating as part of the EURL-AR network, however, the complete list of laboratory codes are known to the EU Commission. Moreover, for laboratories related to the Fleming Fund grant, SEQAFRICA, the complete list of laboratory codes are known to the project management team, Mott MacDonald and the Fleming Fund.

We are looking forward to receiving your results.

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

DTU Genomic PT Coordinator:

Susanne Karlsmose Pedersen
National Food Institute, Technical University of Denmark



Kemitorvet, Building 204,
DK-2800 Kgs. Lyngby, DENMARK
Tel: +45 3588 6601
E-mail: suska@food.dtu.dk

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PROTOCOL for DTU Genomic Proficiency Test, 2020 - APPENDICES

Appendix 1 Using FTP to transfer files

Appendix 2 DTU Genomic PT webtool guideline

Appendix 3 Testforms – overview of method information and results to be submitted via the webtool



Appendix 1

Using FTP to transfer files

FTP is an acronym for File Transfer Protocol and is used to transfer files between computers on a network. To access the folder for upload of files, do as described below.

Obtain access to upload files by using the relevant login provided by the proficiency test organizer.

Using a Windows-computer:

Open the Documents folder, and type 'ftp://cgebase.cbs.dtu.dk/' in the Address bar. Enter you username and password, click "Log on".

Using a Mac-computer:

FileZilla FTP client:

- Download and install FileZilla (<https://filezilla-project.org/>)
- Host:cgebase.cbs.dtu.dk
- Type username and password
- Connect

Or

Finder Mac application:

- In the Finder, choose Go > "Connect to Server," and wait for the pop-up window to show up.
- Specify server address <ftp://cgebase.cbs.dtu.dk> and click "Connect"
- In the new pop-up window enter you username and password, click "Connect"



Appendix 2

DTU Genomic PT webtool guideline

⇒ Browser requirements

IMPORTANT: The system works with the following browsers

Browser	Oldest supported version*
Google Chrome	44.0
Firefox	39.0

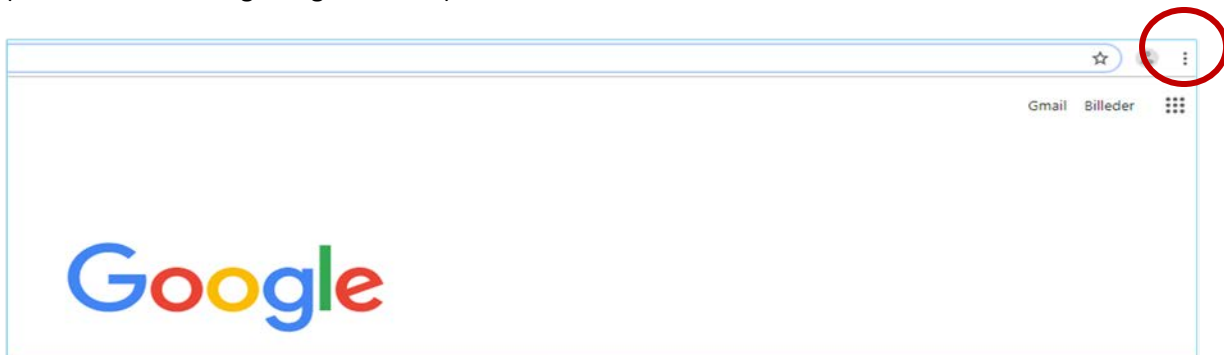
* latest version is recommended

⇒ Access the webtool

IMPORTANT: To access the webtool, you must use an incognito window.

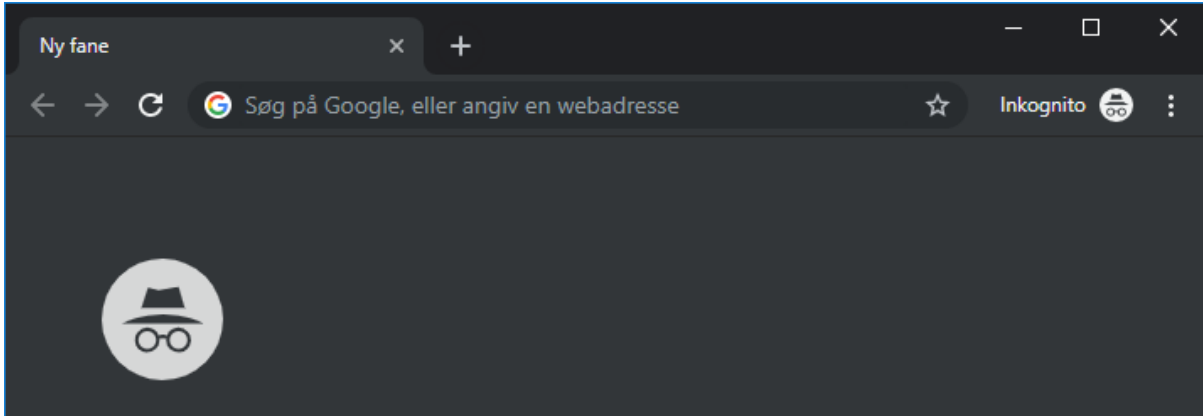
NOTE: Should you have issues with requesting an incognito window, please contact the PT Coordinator (suska@food.dtu.dk) directly.

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

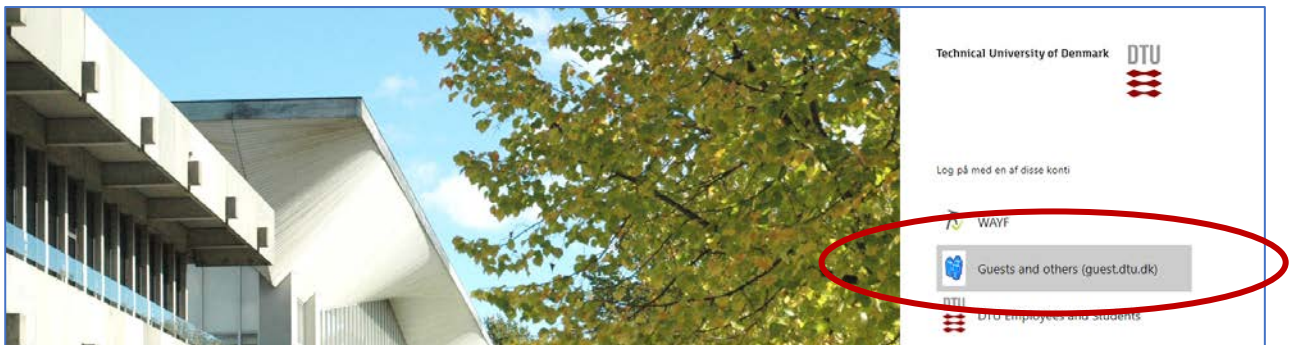




Continue in the black window that looks like this (relevant when using Google chrome):



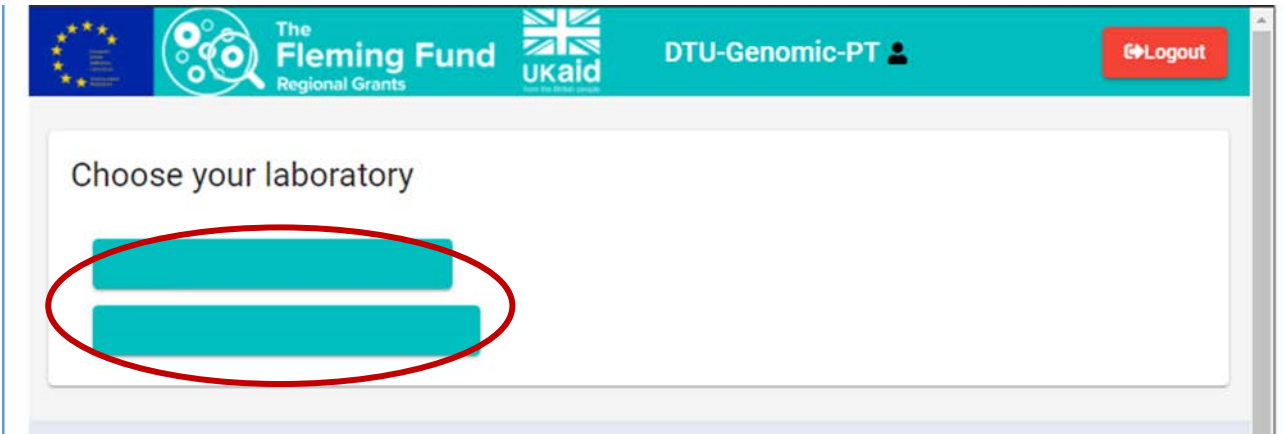
Access the webtool using this address: <https://genomic-pt.dtu.dk>.



Select: Guests and others (guest.dtu.dk).

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

After sign in you will reach the *Proficiency Test Overview* page

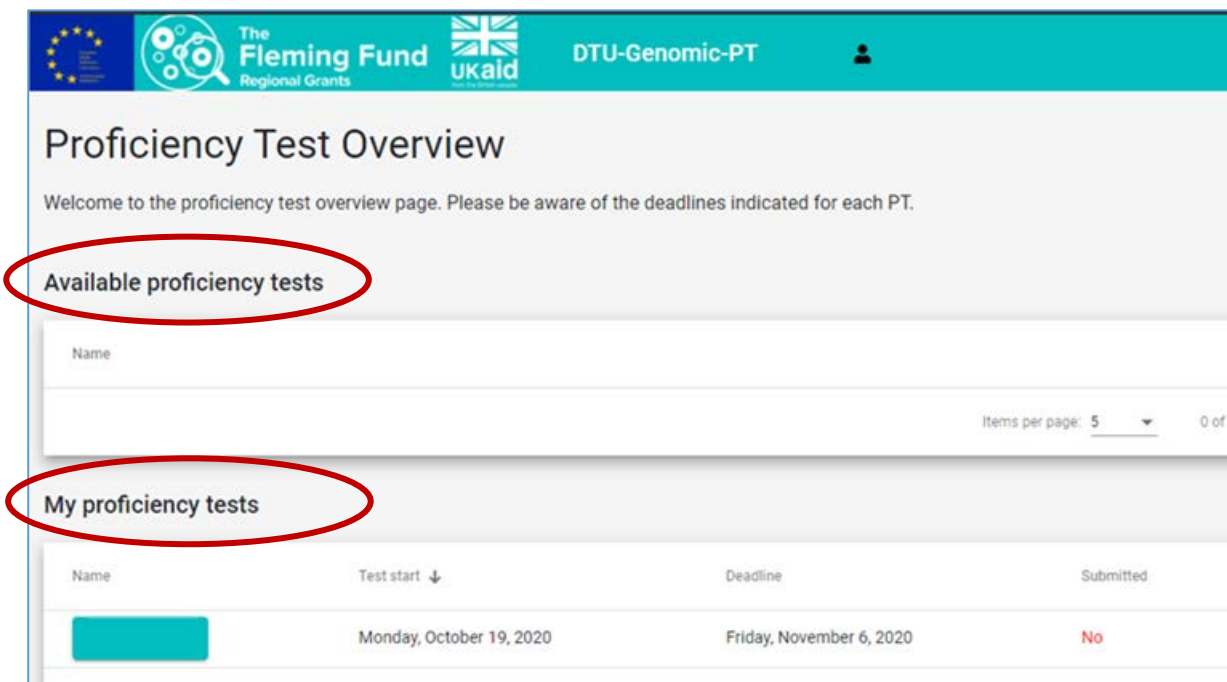


If you are connected to more than one specific laboratory, you will need to select the specific laboratory that you intend to submit results for.

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 proficiency test. Proficiency tests you signed up to will be listed under 'My proficiency tests'.





⇒ Navigate in the webtool

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

- 1 **'About' tab.** Enter data regarding the receipt and the storage PT test material. In this tab, select one, two or three test organisms (*Salmonella*, *E. coli* and *Campylobacter*) that you wish to submit results for
- 2 **'Method' tab.** Enter data regarding processing of the bacterial cultures, the DNA received, DNA purification, sequencing and sequence analysis
- 3 **'AMR' tab.** Enter data regarding resistance genes, chromosomal mutations and predicted phenotype
- 4 **'MLST' tab.** Enter data regarding sequence type and alleles
- 5 **'Serotype' tab.** For *E. coli*, enter data regarding O- and H-antigens. For *Salmonella*, enter data regarding serogroup and serotype. A serotype tab is not available for *Campylobacter*.

⇒ Enter data

Ad 1. 'About' tab

Respond to the general questions (an asterisk (*) indicates that a response is mandatory to complete the proficiency test) and select one, two or three test organisms to proceed. If an organism is selected under the 'About' tab, remaining tabs related to this organism will be activated and allows for accessing and entering results.

The screenshot shows the 'About' tab of the webtool interface. At the top, there are three tabs: 'About', 'Salmonella', 'E. coli', and 'Campylobacter'. The 'About' tab is currently selected. Below the tabs, there is a section titled 'Analysed organism:' with three radio button options: 'Salmonella' (which is selected and circled in red), 'E. coli', and 'Campylobacter'. Below this section, there is a section titled 'General questions'.

Ad 2. 'Method' tab

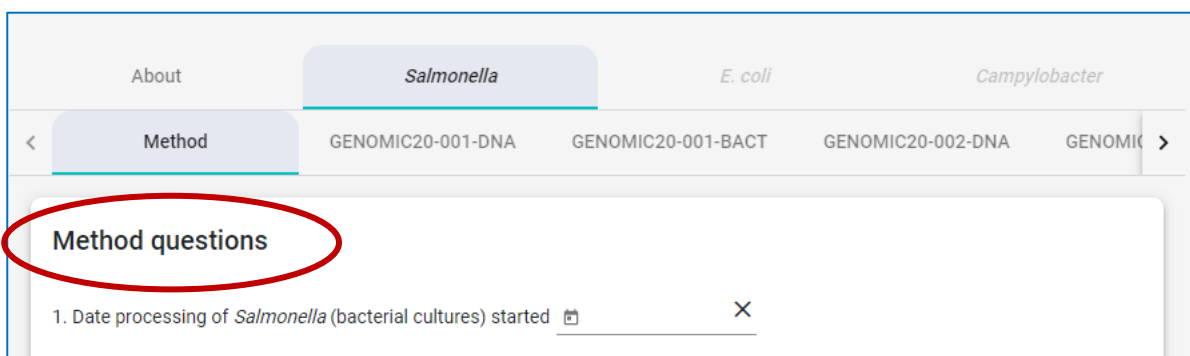
For each of the selected organisms, respond to the questions in the 'Method' tabs. An asterisk (*) indicates that a response is mandatory to complete the proficiency test. Initially, there are some introductory



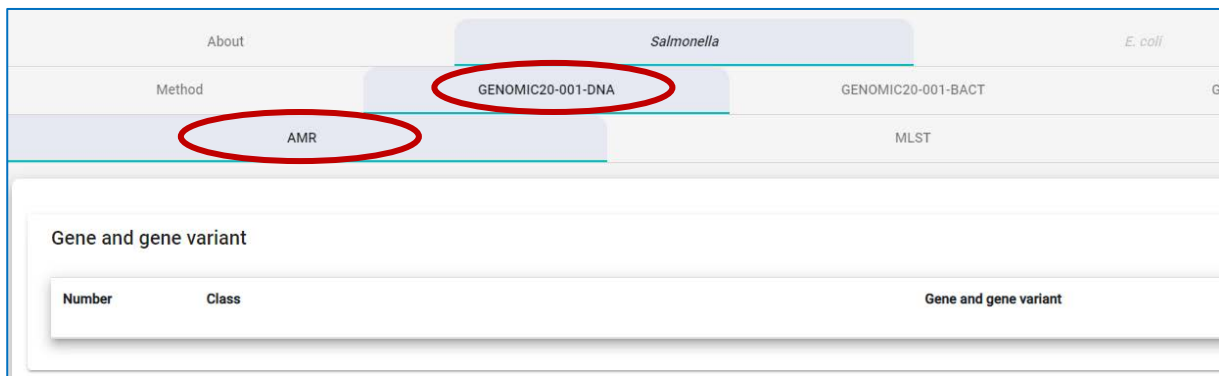
questions regarding growth conditions and DNA extractions methods. Hereafter, the questions are divided into the following sections:

- For the BACTERIAL CULTURES received
- For the DNA received
- SEQUENCING
- ANALYSIS of sequences
- SUBMITTED datafiles

Under the 'Method' tab, when typing numbers, use comma as a separator (i.e. 5,25 and not 5.25).



Select the tab related to one of the test strains (e.g. GENOMIC20-001-DNA). This opens for access to additional tabs.



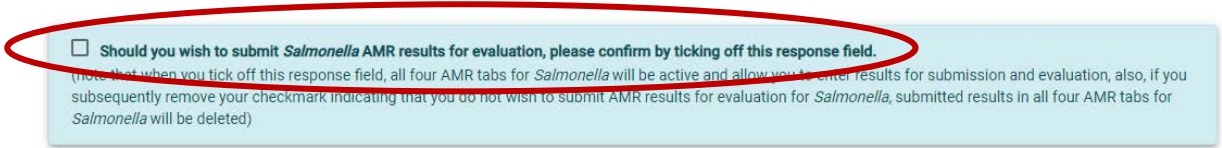
Ad 3. 'AMR' tab:

Under the AMR-tab, results related to 1) identification of antimicrobial resistance genes, 2) chromosomal mutations inducing antimicrobial resistance, 3) upregulated AmpC (relevant for *E. coli*) and subsequently 4) identification of the predicted phenotype of the culture/pre-prepared DNA are uploaded.

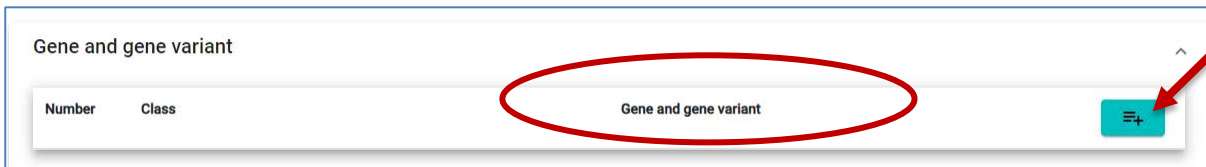
As described in the protocol, it is mandatory to submit AMR results for laboratories part of the SEQAFRICA grant and laboratories belonging to the EURL-AR network. For all other participating laboratories, it is optional to participate in the AMR component.



Therefore, laboratories for which the AMR component is optional will see a question presented in a light blue field: 'Should you wish to submit AMR results for evaluation, please confirm by ticking off this response field' Ticking off the response field will mean that **'Submitted results for all questions related to AMR will be evaluated and scored'**.

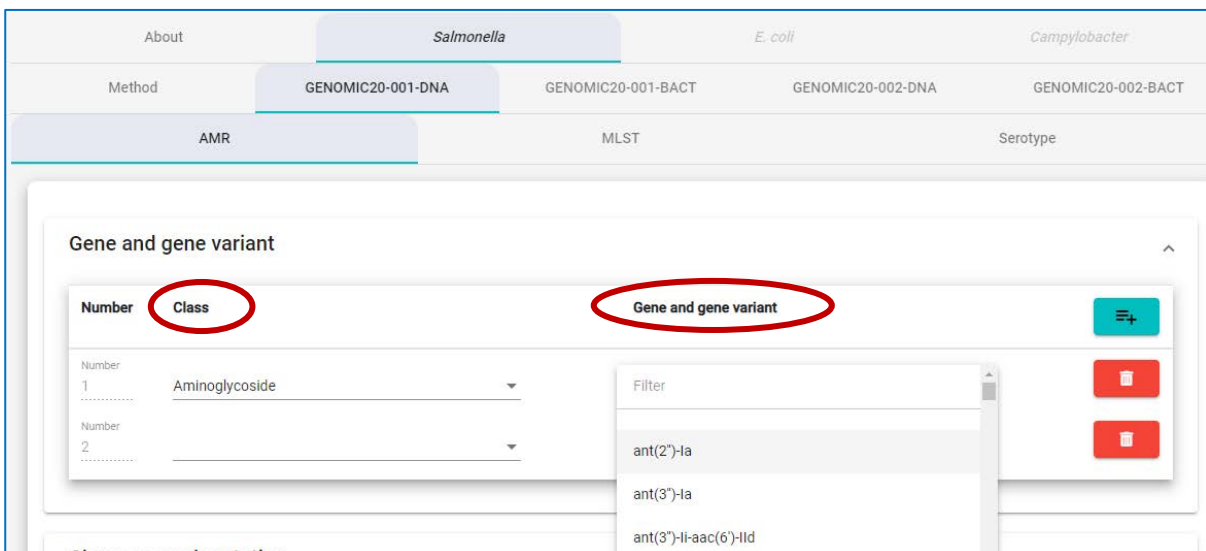


Gene and gene variant: To report genes and gene variants detected in the sequences of the test strains, please click on the '+' (see arrow below) to access dropdown lists.



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

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



Note that antimicrobial resistance towards a limited number of antimicrobial classes are considered in this PT. Antimicrobial classes represented in the DTU Genomic PT, and consequently in the webtool drop down list are:



- Aminoglycoside
- Beta-lactam
- Fluoroquinolone
- Folate pathway antagonist
- Fosfomycin
- Glycopeptide
- Lincosamide
- Macrolide
- Oxazolid
- Oxazolidinone
- Phenicol
- Pleuromutilin
- Polymyxin
- Pseudomonic acid
- Quinolone
- Rifampicin
- Steroid antibacterial
- Streptogramin A/B
- Tetracycline

Note: Ciprofloxacin can here be defined as both a quinolone and a fluoroquinolone.

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. In some fields with multiple classes, the classes have been abbreviated as follows:

- Aminoglycoside: Amino
- Fluoroquinolone: Fluoro
- Lincosamide: Linco
- Macrolide: Macro
- Oxazolidinone: Oxazolid
- Pleuromutilin: Pleuro
- Rifampicin: Rif
- Streptogramin: Stg
- Tetracycline: Tet

All genes conferring resistance to the above-mentioned antimicrobials should be reported, including genes associated with intrinsic resistance.

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



Number	Class	Gene and gene variant	
Number 1	Beta-lactam	blaACC-1	
Number 2			

‘Chromosomal mutations’: In the drop down 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)

Number	Class	Chromosomal mutations	
Number 1	Aminoglycoside, 16S polymyxin, pmrA polymyxin, pmrB Quinolone, gyrA Quinolone, gyrB Quinolone, parC	value	

Example 1: Reporting a mutation in the *pmrA* 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 drop down list under ‘Class’, select the ‘polymyxin, *pmrA*’ option and write G15L in the ‘value’ field under ‘Chromosomal mutations’.

Number	Class	Chromosomal mutations	
Number 1	polymyxin, pmrA	value G15L	

Example 2: If the mutation is in a 16S or a 23S rRNA gene please select the class of antimicrobial and associate gene (e.g. Macrolide, 23S rRNA) from the drop down 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. A523C). Same principle as for the amino acids.

Chromosomal mutation

Number	Class	Chromosomal mutations
Number 1	Macrolide, 23S rRNA	value A523C

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

Chromosomal mutation

Number	Class	Chromosomal mutations
Number 1	Quinolone, parE	value X11X
Number 2		value

‘Upregulated AmpC’: For *E. coli*, 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 drop down 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 PT.

Antimicrobials represented in the DTU Genomic PT, and consequently in the webtool drop down list are:

- Ampicillin
- Azithromycin
- Cefepime
- Cefotaxime
- Cefotaxime+Clavulanic acid
- Cefoxitin
- Ceftazidime
- Ceftazidime+Clavulanic acid
- Chloramphenicol
- Ciprofloxacin
- Clindamycin
- Colistin
- Dalfopristin
- Daptomycin
- Ertapenem
- Erythromycin



- Fusidic acid
- Gentamicin
- Imipenem
- Kanamycin
- Linezolid
- Meropenem
- Mupirocin
- Nalidixic acid
- Penicillin
- Quinupristin
- Rifampicin
- Streptomycin
- Sulfamethoxazole
- Teicoplanin
- Temocillin
- Tetracycline
- Tiamulin
- Tigecycline
- Trimethoprim
- Vancomycin

‘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. Note, however, that these results will *not* be further evaluated.

The screenshot shows a form with a dropdown menu labeled 'Predicted phenotype' and a text area labeled 'Comment'. Below the form is a blue 'Save' button. Red circles highlight the 'Predicted phenotype' dropdown, the 'Save' button, and the 'Comment' text area.

Ad 4. ‘MLST’ tab

Enter data regarding sequence type and alleles. Submission of MLST results (MLST type and corresponding allele numbers) are mandatory.

Ad 5. ‘Serotype’ tab

For *E. coli*, in the dropdown-menu, select the relevant O- and H-antigens, independently. For *Salmonella*, in the dropdown-menu, select the relevant serogroup and subsequently select the relevant serotype. A serotype tab is not available for *Campylobacter*. Submission of serotype results are optional.



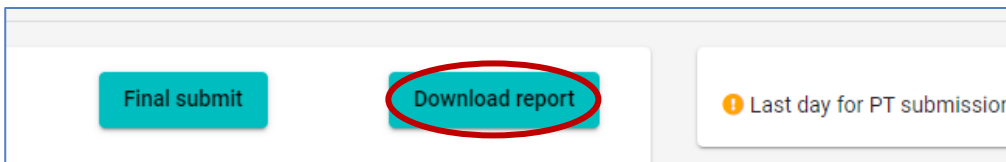
⇒ Save data

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 PT.



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

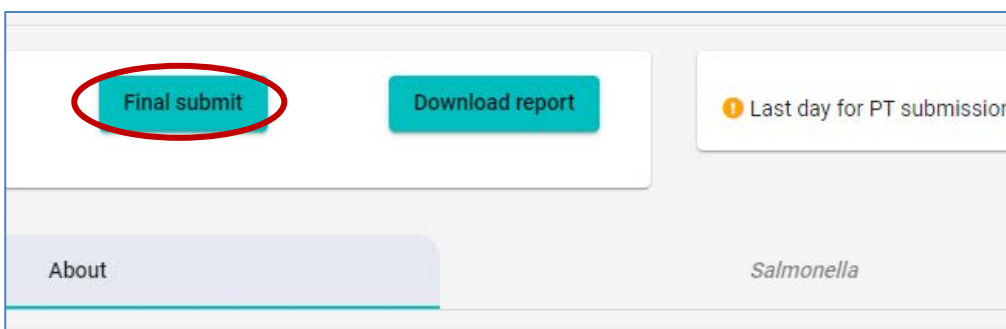
⇒ Submit data

For all organisms of the DTU Genomic PT 2020, all uploaded data are submitted in one go.

When all information and data have been entered and revised for i) the method, and, ii) all three test organisms, 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 will now be 'Yes'

⇒ Evaluation and score

Antimicrobial resistance genes, chromosomal mutations (inducing antimicrobial resistance) and moreover for *E.coli* only, upregulated AmpC identified and reported under a given test strain genome, will be compared to the analysis results based on the respective reference genome. If the obtained and expected results match, a score of '1' will be obtained whereas a mismatch or no-result is scored with '0'.

Similarly, reported predicted phenotypes of the culture/pre-prepared DNA will be evaluated, i.e. if the obtained and expected predicted phenotype matches, a score of '1' will be obtained whereas a mismatch or no-result is scored with '0'.

The reported Multi-locus sequence type (MLST) will be compared to the MLST of the reference genome. A match of the obtained and expected MLST is scored with '1'. A mismatch or no-result is scored with '0'.

The reported O- and H-antigens (*E. coli*) as well as serogroups and -types (*Salmonella*) will be compared to the O- and H-antigens or serogroup and -type of the reference genome, respectively. A match of the obtained and expected O- and H-antigens or serogroup and serotype 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 PT organizer. Upon login to the database, clicking on '*Download report*' will give access to the report presenting obtained results, expected results and scores.

⇒ Support

Should you need support in using the webtool, please do not hesitate to contact the PT 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 PT organizer. Find also link to the DTU Genomic PT website (to access the relevant PT protocol) as well as access to this webtool manual.

Support

PT Organiser: Susanne Karlsmose Pedersen (suska@food.dtu.dk)

[Link to access the PT website](#)

[DTU Genomic PT guideline](#)



Appendix 3

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

With these testforms we invite you to obtain an overview of the data that must/may be submitted to the webtool.

You will find questions in relation to:

1. Sample storage and preprocessing
2. Bacterial Culture; DNA Isolation, Handling and Processing
3. Received DNA; Handling and Processing
4. Sequencing
5. Analysis of sequences; MLST, Serotyping and antimicrobial resistance genes
6. Submitted datafiles

The questions are divided between sections entitled 'About' and 'Method'. In the webtool, the 'About' section has one tab covering all three organisms, whereas the 'Method'-section has a tab for each organism. Therefore, you will find three 'Method' tabs in the webtool and you would need to upload information in each of these, i.e. once per organism that you wish to submit results for.

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

ABOUT

Sample storage and preprocessing

- 1) Date parcel with PT-material was received*:
[DD/MM/YYYY]
- 2) Storage conditions of the bacterial cultures in the time between reception and processing (please select one answer)*:
 - -80°C
 - -20°C
 - 4°C
 - Room temperature
 - No storage time
 - OtherIf other, please define:



- 3) Storage conditions of the DNA in the time between reception and processing (please select one answer)*:
- -80°C
 - -20°C
 - 4°C
 - Room temperature
 - No storage time
 - Other
- If other, please define

METHOD

Note that response to the questions in the 'Method' section below will be requested for each of the organisms that you wish to submit results for.

1. Date processing of bacterial cultures started
2. Date processing of bacterial cultures was completed
3. Date processing of DNA started
4. Date processing of DNA was completed (sequence data submitted)
5. How were the bacterial cultures cultivated*:
 - 5.1. Type of agar media/liquid broth:
 - 5.2. Incubation time (hours):
 - 5.3. Incubation temperature (°C):
6. Which DNA extraction procedure was applied for (enter 'NA' if not relevant)*:
 - 6.1. If manual extraction; kit used, full name:
 - 6.2. If manual extraction; catalogue number of kit:



6.3. If manual extraction, modifications to kit protocol:

6.4. If automatic extraction; robot used:

6.5. If automatic extraction; specific protocol:

6.6. If automatic extraction; modifications to protocol:

Bacterial Culture; DNA Isolation, Handling and Processing

7. For bacterial cultures, how was the DNA concentration (ng/ μ l) prior to library preparation measured (please select one answer)*

- Qubit
- Nanodrop
- DNA concentration not measured
- Other

If other, please define

8. Measurement of DNA concentration (ng/ μ l) for the two test strains (bacterial cultures received)*

For *Salmonella*, GENOMIC20-001-BACT:

For *Salmonella*, GENOMIC20-002-BACT:

For *E. coli*, GENOMIC20-003-BACT:

For *E. coli*, GENOMIC20-004-BACT:

For *Campylobacter*, GENOMIC20-005-BACT:

For *Campylobacter*, GENOMIC20-006-BACT:

9. Measurement of the total DNA amount (microgram) for the two test strains (bacterial cultures received) (not mandatory)

For *Salmonella*, GENOMIC20-001-BACT:

For *Salmonella*, GENOMIC20-002-BACT:

For *E. coli*, GENOMIC20-003-BACT:

For *E. coli*, GENOMIC20-004-BACT:

For *Campylobacter*, GENOMIC20-005-BACT:

For *Campylobacter*, GENOMIC20-006-BACT:



10. For bacterial cultures, how was the DNA quality (e.g. 260/280 ratio and/or 260/230 ratio) prior to library preparation measured (please select one answer) (not mandatory)

- Bioanalyser
- Nanodrop
- DNA quality not measured
- Other

If other, please define

11. If relevant, following your response to the previous question, measurement of DNA quality (e.g. Bioanalyser, 260/280 ratio, other) for the two test strains (bacterial cultures received)

For *Salmonella*, GENOMIC20-001-BACT:

For *Salmonella*, GENOMIC20-002-BACT:

For *E. coli*, GENOMIC20-003-BACT:

For *E. coli*, GENOMIC20-004-BACT:

For *Campylobacter*, GENOMIC20-005-BACT:

For *Campylobacter*, GENOMIC20-006-BACT:

12. If relevant, measurement of DNA quality (260/230 ratio) for the two test strains (bacterial cultures received) (not mandatory)

For *Salmonella*, GENOMIC20-001-BACT:

For *Salmonella*, GENOMIC20-002-BACT:

For *E. coli*, GENOMIC20-003-BACT:

For *E. coli*, GENOMIC20-004-BACT:

For *Campylobacter*, GENOMIC20-005-BACT:

For *Campylobacter*, GENOMIC20-006-BACT:

Received DNA; Handling and Processing

13. For DNA received, how was the DNA concentration (ng/μl) prior to library preparation measured (please select one answer)*

- Qubit
- Nanodrop
- DNA concentration not measured
- Other

If other, please define



14. Measurement of the DNA concentration (ng/ μ l) for the two test strains (for the DNA received) *

For *Salmonella*, GENOMIC20-001-DNA:

For *Salmonella*, GENOMIC20-002-DNA:

For *E. coli*, GENOMIC20-003-DNA:

For *E. coli*, GENOMIC20-004-DNA:

For *Campylobacter*, GENOMIC20-005-DNA:

For *Campylobacter*, GENOMIC20-006-DNA:

15. Measurement of the total DNA amount (microgram) for the two test strains (for the DNA received) (not mandatory)

For *Salmonella*, GENOMIC20-001-DNA:

For *Salmonella*, GENOMIC20-002-DNA:

For *E. coli*, GENOMIC20-003-DNA:

For *E. coli*, GENOMIC20-004-DNA:

For *Campylobacter*, GENOMIC20-005-DNA:

For *Campylobacter*, GENOMIC20-006-DNA:

16. For (DNA received), how was the DNA quality (e.g. 260/280 ratio and/or 260/230 ratio) prior to library preparation measured (please select one answer) (not mandatory)

- Bioanalyser
- Nanodrop
- DNA quality not measured
- Other

If other, please define

17. If relevant, following your response to the previous question, measurement of DNA quality (e.g. Bioanalyser, 260/280 ratio, other) for the two test strains (for DNA received)

For *Salmonella*, GENOMIC20-001-DNA:

For *Salmonella*, GENOMIC20-002-DNA:

For *E. coli*, GENOMIC20-003-DNA:

For *E. coli*, GENOMIC20-004-DNA:

For *Campylobacter*, GENOMIC20-005-DNA:

For *Campylobacter*, GENOMIC20-006-DNA:



18. If relevant, measurement of DNA quality (260/230 ratio) for the two test strains (for DNA received) (not mandatory)

For *Salmonella*, GENOMIC20-001-DNA:

For *Salmonella*, GENOMIC20-002-DNA:

For *E. coli*, GENOMIC20-003-DNA:

For *E. coli*, GENOMIC20-004-DNA:

For *Campylobacter*, GENOMIC20-005-DNA:

For *Campylobacter*, GENOMIC20-006-DNA:

19. Did you perform quality check to verify the quality of the DNA on a gel (yes/no) (see description in the protocol of this optional check)

For *Salmonella*, GENOMIC20-001-DNA:

For *Salmonella*, GENOMIC20-002-DNA:

For *E. coli*, GENOMIC20-003-DNA:

For *E. coli*, GENOMIC20-004-DNA:

For *Campylobacter*, GENOMIC20-005-DNA:

For *Campylobacter*, GENOMIC20-006-DNA:

SEQUENCING

20. Which protocol was used to prepare the sample library for sequencing? For commercial kits please provide the full kit name, item number, and lot number if possible. For noncommercial kits please provide a citation for the protocol, or submit a summary of the protocol. Please note any deviations from the kit or cited protocol (enter 'NA' if not relevant)*:

20.1. For commercial kits; full kit name:

20.2. For commercial kits; catalogue number:

20.3. For commercial kits; lot number:

20.4. For noncommercial kits; citation for the protocol:

20.5. For noncommercial kits; summary of the protocol:

20.6. Deviations from the kit or cited protocol

21. For (organism x, e.g. *Salmonella*), please indicate the sequencing platform you used in the proficiency test (please select one answer)*

- Ion Torrent PGM
- Ion Torrent Proton
- Genome Sequencer Junior System (454)
- Genome Sequencer FLX System (454)
- Genome Sequencer FLX+ System (454)



- PacBio RS
- PacBio RS II
- HiScanSQ
- HiSeq 1000
- HiSeq 1500
- HiSeq 2000
- HiSeq 2500
- HiSeq 4000
- Genome Analyzer Iix
- MiSeq
- MiSeq Dx
- MiSeq FGx
- ABI SOLiD
- NextSeq
- MinION (ONT)
- other

If other, please define

22. Sequencing details #1 (please select one answer)*

- Single-end
- Paired-end
- Not relevant

23. Sequencing details #2:

For the sequencing, the read length (bp) was set to be (expected read length)

24. Reads trimmed before upload (please select one answer)*

[Note; this question refers to trimming performed actively by the participant (i.e. trimming performed automatically by your sequencing machine is not relevant for this question). Ideally, no trimming should be performed.

As part of the analysis of the sequences subsequent to the deadline of the PT, trimming will be performed by application of the same tool for all submitted sequences.

Should trimming be an integrated part of your sequencing process (disregarding possible automatic trimming by your sequencing machine), please indicate with 'yes' to this question]

- Yes
- No

25. If trimmed, which tool was applied (in the following text field, please insert name and URL/link (if possible))



26. For the analysis of the sequences from the bacterial cultures and the corresponding DNA in the proficiency test, assembly is not required. If, however, you were to assemble your sequences, which assembly tool would you apply?
Please insert name and URL (e.g. Velvet, <https://www.ebi.ac.uk/~zerbino/velvet/>, open access)

ANALYSIS of sequences

27. If any, which method was used to characterize or differentiate isolates (please select all that apply)?*
- MLST
 - Allele-based
 - Gene-by-gene-based
 - SNP-based
 - None

If other, please define

28. If you determined the MLST-type of the sequenced DNA, how was the analysis performed (please select one answer)?*

- MLST-analysis was performed on raw reads
- MLST-analysis was performed on contigs
- MLST-analysis was not performed

29. If you determined antimicrobial resistance (AMR) genes present in the sequenced DNA, how was the analysis performed (please select one answer)?*

- Analysis for AMR-genes was performed on raw reads
- Analysis for AMR-genes was performed on contigs
- Analysis for AMR-genes was not performed

30. For the detection of the Multi Locus Sequence Type, which tool did you apply? Please insert name and URL (e.g. MLST 1.7 (MultiLocus Sequence Typing), <http://cge.cbs.dtu.dk/services/MLST/>, open access) (enter 'NA' if not relevant)*:

31. For the detection of the resistance genes harboured in the sequences, which tool did you apply? Please insert name and URL (e.g. ResFinder, <http://cge.cbs.dtu.dk/services/ResFinder/>, open access) (enter 'NA' if not relevant)*:



32. For the detection of serogroup/serotype (not relevant for *Campylobacter*), which tool did you apply? Please insert name and URL (e.g. ResFinder, <http://cge.cbs.dtu.dk/services/ResFinder/>, open access) (enter 'NA' if not relevant):

SUBMITTED datafiles

33. Has the obtained non-assembled sequence data been uploaded for bacterial cultures and DNA following the description in the PT-protocol?* (yes/no)