Appendix 3

Test Forms – overview of method information and results of *in silico* analyses to be submitted via the DTU Webtool

These test forms are provided to give an overview of the data that must or may be submitted to the webtool.

You will find questions related to:

1. Sample storage and preprocessing
2. Bacterial Culture, DNA Extraction, Handling and Processing
3. Sequencing
4. Analysis of sequences: MLST, Plasmid replicons, and antimicrobial resistance mechanisms
5. Submitted datafiles

Below, you find questions divided into 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. Although the questions in this document are compiled for all species, in the webtool you will find *three separate 'Method' tabs*, one for each microorganism. You must upload the relevant information for each organism you are submitting results for.

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

ABOUT

Sample storage and preprocessing

1) Date the parcel with PT-material was received*:

   [DD/MM/YYYY]

2) Storage conditions of the bacterial cultures between reception and processing (select one answer)*:
   - -80˚C
   - -20˚C
   - 4˚C
   - Room temperature
   - No storage
   - Other

If other, please specify:

METHOD

Please note that the following questions in the 'Method' section must be answered for each organism for which you are submitting results.

1. How were the bacterial cultures cultivated*:

   a) Type of agar media/liquid broth:
   E. coli:
   S. aureus:
   Enterococcus:

   b) Incubation time (hours):
   E. coli:
   S. aureus:
   Enterococcus:

   c) Incubation temperature (˚C):
   E. coli:
   S. aureus:
   Enterococcus:

2. Please provide information about the DNA extraction procedure used and indicate any modifications to the kit protocol.

   a) If manual extraction; kit used (full name):
   E. coli:
   S. aureus:
   Enterococcus:

   b) If manual extraction; catalogue number of kit:
   E. coli:
   S. aureus:
   Enterococcus:

   c) If manual extraction, modifications to kit protocol:
   E. coli:
   S. aureus:
   Enterococcus:

   d) If automatic extraction; robot used:
   E. coli:
   S. aureus:
   Enterococcus:

e) If automatic extraction; specific protocol:
E. coli:
S. aureus:
Enterococcus:

f) If automatic extraction; modifications to protocol:

E. coli:
S. aureus:
Enterococcus:

For BACTERIAL CULTURES received

3. Method used to measure DNA concentration after the extraction? (please select one answer)*

- Qubit® (Invitrogen™/Thermo Fisher Scientific)
- NanodropTM (Thermo Fisher Scientific)
- BioanalyzerTM (Agilent Technologies)
- DNA concentration not measured
- Other

If other, please specify:

4. DNA concentration (ng/µl) after extraction, for each test strain*

For *E. coli*, GPT24-01:
For *E. coli*, GPT24-02:
For *S. aureus*, GPT24-03:
For *S. aureus*, GPT24-04:
For *Enterococcus*, GPT24-05:
For *Enterococcus*, GPT24-06:

5. Total DNA amount (microgram) after extraction, for each test strain (not mandatory)

For *E. coli*, GPT24-01:
For *E. coli*, GPT24-02:
For *S. aureus*, GPT24-03:
For *S. aureus*, GPT24-04:
For *Enterococcus*, GPT24-05:
For *Enterococcus*, GPT24-06:

6. Method used to evaluate the DNA quality after extraction (*e.g.* 260/280 ratio and/or 260/230 ratio) (please select one answer) (not mandatory)

- Bioanalyser
- Nanodrop
- DNA quality not measured

- Other

If other, please specify

7. Measurement of DNA quality after extraction (260/280 ratio) for each test strain (if relevant) (not mandatory)

   For *E. coli*, GPT24-01:
   For *E. coli*, GPT24-02:
   For *S. aureus*, GPT24-03:
   For *S. aureus*, GPT24-04:
   For *Enterococcus*, GPT24-05:
   For *Enterococcus*, GPT24-06:

8. Measurement of DNA quality after extraction (260/230 ratio) for each test strain (if relevant) (not mandatory)

   For *E. coli*, GPT24-01:
   For *E. coli*, GPT24-02:
   For *S. aureus*, GPT24-03:
   For *S. aureus*, GPT24-04:
   For *Enterococcus*, GPT24-05:
   For *Enterococcus*, GPT24-06:

SEQUENCING

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

   a) For commercial kits; full kit name:
   b) For commercial kits; catalogue number:
   c) For noncommercial kits; citation for the protocol:
   d) For noncommercial kits; summary of the protocol:
   e) Deviations from the kit or cited protocol:

10. Please indicate the sequencing platform you used in the proficiency test (please select one answer)*

- HiSeq® (Illumina Inc., California, USA)
- Ion Torrent™ Platforms (Thermo Fisher Scientific, Massachusetts, USA)
- MGI DNBSEQ Series (MGI Tech, Shenzhen, China)
- MiSeq® (Illumina Inc., California, USA)
- MiniSeq® (Illumina Inc., California, USA)
- NextSeq® (Illumina Inc., California, USA)
- NovaSeq® (Illumina Inc., California, USA)
- Other

If other, please specify:

Please specify the model/version of the platform you used (e.g., NextSeq® 550):

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

- Single-end
- Paired-end

12. Sequencing details #2:

What was the expected read length (in base pairs) based on the sequencing platform you used?

13. How was the quality control (QC) of the raw sequencing data performed? (e.g., FASTQC, other)?

14. Were the reads trimmed before upload? (please select one answer)*:

(**Note**: This refers to trimming performed manually by the participant or as part of an integrated pipeline, not trimming automatically done by the sequencing machine). Ideally, trimming should not be performed before upload.

- Yes
- No

15. If the reads were trimmed, which tool was used (please provide the tool name and URL/link if possible)

16. If applicable, which assembly tool did you use to assemble the reads? Please provide the tool name, version number and URL (e.g., SPAdes, version 3.15.4, https://cab.spbu.ru/software/spades/)

17. If applicable, how was the QC of the assemblies performed? Please mention the program (e.g., QUAST) and the QC metrics (e.g. N50, L50 etc.) used.

ANALYSIS of sequences

18. For the Multilocus Sequence Typing (MLST) of the sequenced DNA, how was the analysis performed? (please select one answer) *

- MLST was performed on raw reads
- MLST was performed on contigs
- MLST was performed on both

19. For determining the plasmid replicons in the sequenced DNA, how was the analysis performed? (please select one answer) *

- Plasmid replicons were determined on raw reads
- Plasmid replicons were determined on contigs
- Plasmid replicons was determined on both

20. For determining antimicrobial resistance (AMR) mechanisms (genes, mutations) in the sequenced DNA, how was the analysis performed (please select one answer)?*

- Analysis for AMR mechanisms was performed on raw reads
- Analysis for AMR mechanisms was performed on contigs
- Analysis for AMR mechanisms was performed on both

21. For the Multilocus Sequence Typing (MLST), 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, the version number and the URL. 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

22. For the detection of plasmid replicons, 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, the version and the URL. If you used an in-house script, please specify the program
c) Database: publicly available, commercial, or in-house database. If you used an available database, please report the name and version number. If you used an in-house database, please briefly describe the genes included.
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.)

23. 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, the version and the URL. If you used an in-house script, please specify the program
c) Database: publicly available, commercial, or in-house database. If you used an available database, please report the name and version number. If you used an in-house database, please briefly describe the genes included.

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.)

24. 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, the version number and the URL. If you used an in-house script, please specify the program
c) Database: publicly available, commercial, or an in-house database. If you used an available database, please report the name and version number. If you used an in-house database, please briefly describe the point mutations included.
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.)

25. 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, the version number and the URL. If you used an in-house script, please specify the program
c) Database: publicly available, commercial, or an in-house database. If you used an available database, please report the name and version number. If you used an in-house database, please briefly describe the sequences included
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.)

SUBMITTED data files

26. Have the FASTQ files (non-assembled sequence data, file names as indicated in the protocol) been uploaded to the ScienceData folder for bacterial cultures following the description in the PT protocol? Please confirm by ticking off the response field* (yes/no)