





PROTOCOL for GMI Proficiency Test, 2016

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1 OVERVIEW AND OBJECTIVES

The proficiency test, 2016, consists of three general parts:

- 1a. DNA extraction, purification, library-preparation, and whole-genome-sequencing from **live cultures**
- 1b. Whole-genome-sequencing of pre-prepared DNA
- 2. Phylogenetic/clustering analysis of three fastq datasets

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. phylogeny). This ensures that the discrepancies and differences among laboratories are known and will contribute to the standardization of whole genome sequencing and data analysis, with the aim to produce comparable data for the GMI initiative. A further objective is to assess and improve the uploaded data to databases such as NCBI, EBI and DDBJ.









The GMI proficiency test 2016 is supported by COMPARE, which has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 643476. In addition, the GMI PT 2016 is supported by the GenomeTrakr Network and Microbiologics[®].

2 INTRODUCTION

GMI is a global, visionary taskforce of scientists and other stakeholders who share an aim of applying novel genomic technologies and informatics tools to improve global patient diagnostics, surveillance and research, by developing needs- and end-user-based data exchange and analysis tools for characterization of all microbial organisms and microbial communities.

The GMI working group 4 (WG4) steered by the US FDA, Microbiologics, and Technical University of Denmark has prepared this proficiency test (PT). The PT consists of three parts, each of which are optional, and include assessing (1a) the laboratory's DNA preparation and sequencing procedures, (1b) the laboratory's sequencing output, and (2) the laboratory's procedure to identify variant sites within whole genome sequence data and cluster and distinguish samples based on those variants.

The proficiency test focuses on *Campylobacter jejuni*, *Listeria monocytogenes* and *Klebsiella pneumonia*, and allows for sign-up for each species separately. Note that item 1a and item 1b are parallel; i.e. when signing up for 1a for one species, the participation in 1b is also expected.

The three items consist of

1a) DNA extraction, purification, library-preparation, and whole-genome-sequencing of six bacterial cultures: two *Campylobacter* strains (one is *C. coli* and one is *C. jejuni*), two *Listeria monocytogenes* strains and two *Klebsiella pneumonia* strains. Participants will be requested to upload reads to an ftp-site and **optionally** also identify the Multi Locus Sequence Type (MLST) of the strains as well as the resistance genes present in the strains if that is something that is routinely done within the laboratory.

1b) Whole-genome-sequencing of pre-prepared DNA delivered by GMI Working Group 4 of the same six bacterial strains mentioned in clause 1a.

2) Variant detection and phylogenetic/clustering analysis of three datasets each including fastq data from circa 20 genomes of *C. jejuni, L. monocytogenes*, and *K. pneumonia*. Note: If performing a reference based approach for variant detection, the reference applied for the analysis must be the species specific references indicated below (see 3.4.2).









Institutes/organizations which signed up to participate will receive the PT-material (bacterial strains, DNA and/or the login for download of datasets) according to the registered sign-up information.

3 OUTLINE OF THE GMI PT

3.1 Shipping, receipt and storage of bacterial strains

In October 2016, around 60 laboratories located worldwide will receive a parcel containing two strains of each *Campylobacter, L. monocytogenes,* and *K. pneumonia* together with corresponding purified DNA (according to the registered sign-up information). All bacterial strains and DNA are shipped as UN3373, Biological substance category B. Those who signed up for item 2 (phylogenetic analysis) will receive information and login for downloading the three datasets.

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

The bacterial strains are shipped lyophilised as KwikStik's (see below for additional info on handling). On arrival, the KwikStik's must be refrigerated until handling in the laboratory.

The bacterial DNA is shipped as dried samples using a DNA stabilizing agent (DNAstable[®] *Plus*, Biomatrica). On arrival, either rehydrate your sample 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 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. Should moisture start to appear, the desiccant pack must be changed.

3.2 Using FTP to transfer files

For download of fastq files for item 2 and for upload of results, an ftp-server is used. The proficiency test organizer will provide each participant with username and login for this purpose. The ftp-site which will be used for this purpose is cgebase.cbs.dtu.dk. For information on how to transfer files, please see Appendix 1.

3.3 Supplied test material

3.3.1 Item 1a; Bacterial cultures

The procedure for reconstitution of the bacterial cultures should follow the manufacturer's procedures as presented in the instructional video or the written instructions on their website (see









http://microbiologics.com/s.nl/sc.7/category.98564/.f or http://microbiologics.com/Support-Center/KWIK-STIK-trade).

MSDS for KwikStik are found here: <u>http://microbiologics.com/Support-Center/Lyophilized-Microorganism-Preparations</u>.

The bacterial cultures supplied have been sequenced multiple times and the genomes have been closed. Therefore, the PT-organizers encourage participants to maintain these bacterial strains in their strain collection and apply them as part of future internal quality control.

3.3.2 Item 1b; DNA

The supplied DNA has been stabilized by DNA Stable[®]plus (<u>https://www.biomatrica.com/media/dnastable%20Plus/3001-0313.pdf</u>). Each vial contains a minimum of 2 µg DNA.

Before use, the DNA should be rehydrated. Add $60 - 100 \mu$ l 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 be used directly in downstream application.

Unused rehydrated sample can be stored for up to one month at 4 degrees or room temperature.

Optional: The quality of the rehydrated DNA can be checked, by running it on a gel. The amount of DNA supplied in each tube will be sufficient to allow running a small fraction on a gel.

3.3.3 Item 2; Fastq data set

Three datasets, one for each of *C. jejuni* (cj), *L. monocytogenes* (Im), and *K. pneumonia* (kp) will be available for download from the ftp-site 'cgebase.cbs.dtu.dk'. Login to the ftp-site will be provided directly to each participant. Each dataset will consist of the original fastq files (i.e., whole genome sequence data) from circa 20 samples for phylogenetic cluster analysis based on a tool of the laboratory's own choice; SNP-calling, gene-by-gene, etc.

3.4 Procedure and analysis of test material

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

Subculture the bacterial strains on a relevant growth medium of the laboratory's own choice and incubate. Following incubation and assessment of purity of the bacterial cultures, perform DNA extraction and whole-genome-sequencing according to the laboratory's standard procedure.









For the purified PT-DNA received, perform whole-genome-sequencing according to the laboratory's standard procedure.

For both bacterial cultures and DNA (items 1a and 1b), register relevant information related to the methods applied via <u>https://www.surveymonkey.com/r/PT 2016 bacterial cultures and DNA</u> (also see Appendix 2). Appendix 2 also describes the requested results when analyzing the sequences as regards the detected antimicrobial resistance genes and as regards the Multi Locus Sequence Type of the bacterial strain.

3.4.2 Item 2; Fastq data set

The three fastq datasets should be downloaded from the ftp-site. They are organized into three different .zip archives appropriately labeled with the taxon they represent (cj, lm, or kp). Within each archive the participant will find the paired-end reads. The objective associated with this dataset is to assess the variability of laboratories in the clusters identified, which may be part of routine traceback investigations and/or source-tracking, through the analysis of next-generation sequencing data. As such, the participant should employ their preferred method for constructing a matrix (e.g., gene, SNP, presence/absence, etc.) and for clustering samples (e.g., distance-, maximum-likelihood-, Bayesian-based).

If performing a reference based approach for variant detection, the reference applied for the analysis **must be** cj_reference.fasta (*C. jejuni*), Im_reference.fasta (*L. monocytogenes*) and kp_reference.fasta (*K. pneumonia*).

4 DISCUSSION FORUM

A web-based discussion forum (<u>https://foros.isciii.es/viewforum.php?f=7</u>) is available for participants in the GMI PT 2016, allowing for individual sign-up and discussion with other PT-participants and the PT organizers in relation to issues relating to the analysis for the present PT. Appendix 4 presents detailed information on the PT discussion forum. We strongly encourage the use of this forum as both a resource among participants but also as a platform to ask questions of the organizers – which other participants might also be interested in hearing the answer.

5 REPORTING OF RESULTS AND EVALUATION

For all items (1a, 1b and 2), the results should be captured and entered into the Internet-based survey (<u>https://www.surveymonkey.com/r/PT_2016_bacterial_cultures_and_DNA</u> and <u>https://www.surveymonkey.com/r/PT_2016_FASTQ_dataset</u>). See also Appendix 2 and 3.









5.1 Procedure and analysis of test material

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

Results for item 1a and 1b must be submitted as a batch-upload. The web-interface of the batch upload interface (<u>https://cge.cbs.dtu.dk/services/ringtrials/</u>) provides a possibility to upload several isolates in a single submission. The interface is divided into five steps, and a progress bar presents the overview of the submitted files.

Step 1; Login to the server using provided username (gmi_xx) and password (pink area).

<u>Step 2</u>; Download the Excel Metadata template to your computer (green area).

<u>Step 3</u>; Fill in the required fields with all the relevant information (metadata) about the isolates, the associated WGS file names, sequencing platform and sequencing type used to generate the data, etc. Each cell has a brief description of the required metadata. Some of the fields provide drop-down lists with possible metadata. Note that **sample name** should be the **same as label-name of the sample**, e.g. **GMI16-003-BACT** or **GMI16-003-DNA**.

<u>Step 4</u>; When the spreadsheet is properly filled out, upload the metadata file as well as the individual WGS files, that were included as metadata in the spreadsheet, to the web-interface by dropping the files to the 'Drop metadata and sequence files here' (grey area). After this, the spreadsheet will be validated to check if the metadata has the correct format.

If the spreadsheet contains invalid metadata, an error message will appear at the top of the uploader (between blue and green). To fix the errors, correct the errors in the original spreadsheet and upload the updated spreadsheet.

<u>Step 5</u>; Click on the Submit button to upload the files. The progress will be displayed in the Upload Progress bar (below the blue 'Submit' button). It is important to keep the window opened until the upload is completed. Pre-loading and validation will be displayed in blue and uploading – in green. After that, the web-interface will automatically submit the job to the server.

If the job is submitted correctly, you will get an on-screen confirmation message.

Via the Internet-based survey

(<u>https://www.surveymonkey.com/r/PT_2016_bacterial_cultures_and_DNA</u>; see also Appendix 2), answers should be submitted to the questions related to the analysed bacterial cultures and DNA.









5.1.2 Item 2; Fastq data set

For the fastq data set analyses, three types of files should be submitted and please pay close attention to the instructions on how samples and files should be named and which samples to include.

For each dataset:

- 1. The DNA sequence matrix used for clustering should be in fasta format and have that as the file extension
 - The matrix should only contain **only** those samples provided through the ftp site (i.e., there should be only 15 cj, 20 lm, and 17 kp samples in each file no more no less). Please ensure that the fasta alignment has the exact number required by counting the number of sequence header prefixes (>).
 - Syntax for the names of samples in the matrix should be *only* the prefix preceding the first underscore in the file name. For example, **cj1_1.fastq** should be named **cj1** in the matrix.
 - The file should be named as follows GMILabID_Taxon.fasta (e.g., GMI01_cj.FASTA, GMI01_lm.FASTA, or GMI01_kp.FASTA).
- 2. The clusters themselves in newick format with the .tre as the file extension
 - Again, the tree should only contain those samples provided through the ftp site
 - Syntax for the names of samples in the tree file should be *only* the prefix preceding the first underscore in the file name. For example, **cj1_1.fastq** should be named **cj1** in the matrix.
 - The file should be named as follows GMILabID_Taxon.tre (e.g., GMI01_cj.TRE, GMI01_lm.TRE, or GMI01_kp.TRE).
 - Ideally the tree files would include branch lengths but this is not required.
- 3. The vcf (variant call format) files for each sample if a reference based approach was used and such files were produced.
 - The number of vcf files should match the number of samples found in the zipped archive from the ftp site.
 - Syntax for the names of the files should be *only* the prefix preceeding the first underscore in the file name. For example, **cj1_1.fastq** should be named **cj1** in the matrix.
 - The file should be named as follows GMILabID_Taxon.tre (e.g., GMI01_cj1.vcf, GMI01_lm1.vcf, or GMI01_kp1.vcf).

Via the Internet-based survey (<u>https://www.surveymonkey.com/r/PT_2016_FASTQ_dataset</u>; see also Appendix 3), answers should be submitted to the questions related to the Fastq data set section.









5.2 Evaluation of results

For both bacterial cultures and DNA (items 1a and 1b), the submitted sequence data (fastq-files) will be assembled using SPAdes [http://bioinf.spbau.ru/spades] and evaluated according to the following specific quality markers: e.g. read length (bp), N50 (bp), total number of contigs and total length of sequence (bp) including percentage of reference genome covered. In addition, the PT-organizers will assemble the submitted reads and compare these assemblies 1) towards the relevant closed genome to assess the sequence error rate and coverage of the scaffold and 2) between the obtained sequences in items 1a and 1b.

Assessment of the submitted results from the analysis of the fastq datasets (item 2) is based on two criteria: 1) the concordance among laboratories in their answers to the questions in the SurveyMonkey (Appendix 3) and 2) the concordance between participants' in the information content contained in the SNP-matrix, vcf-files and the relationships among samples from the clustering analyses (i.e., the topology).

For the evaluation of the results, no official GMI quality threshold is currently available and therefore no acceptance limit has been defined for this proficiency test.

5.3 Deadline for submission of results

Results must be submitted electronically **no later than December 14th, 2016**. Immediately after this date, the survey will be closed and results submitted to the Internet-based survey, via the batch-upload and to the ftp-site will be evaluated. Delayed submission of results will not be accepted.

5.4 Analysis and publication of results

Individual results will be anonymized, and only the PT-organizers will have access to your laboratory's results. Each participating laboratory will receive an individual summary of the obtained performance. An overall report summarizing the results will be published and 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.

We are looking forward to receiving your results.









If you have any questions or concerns, please do not hesitate to contact us, preferably by using the web-based discussion forum (<u>https://foros.isciii.es/viewforum.php?f=7</u>). In addition to receiving a response to your question, bringing up an issue via the forum allows other participants to also benefit from the discussions and the PT-organizers' response.

PT organizer related to the dry-lab fastq datasets:

James Pettengill U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition CPK1 RM2D0195100 Paint Branch Parkway College Park, MD 20740, US Tel: +1 240-402-1992 E-mail: James.Pettengill@fda.hhs.gov

PT organizer in relation to other issues, e.g. organizational issues, please contact the EQAS Coordinator:

Susanne Karlsmose Pedersen National Food Institute, Technical University of Denmark Søltofts Plads, Building 221, room 238, DK-2800 Kgs. Lyngby, DENMARK Tel: +45 3588 6601 E-mail: <u>suska@food.dtu.dk</u>







PROTOCOL for GMI Proficiency Test, 2016 - APPENDICES

- Appendix 1: Using FTP to transfer files
- Appendix 2: Overview of Internet-based survey bacterial cultures and DNA
- Appendix 3: Overview of Internet-based survey FASTQ dataset
- Appendix 4: GMI Proficiency Test Forum guide







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 or download of files, do as described below.

Obtain access to upload or download 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 (<u>https://filezilla-project.org/</u>)
- 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"

Introduction

This survey seeks to capture info on participants' sequence procedures and specifications in relation to the bacterial cultures and DNA tested as part of the GMI Proficiency Test (PT) 2016.

The survey consists of six sections, collecting information on

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

If you have any questions or feedback for the submission of data via this survey, please contact the PT Coordinator, Susanne Karlsmose Pedersen (suska@food.dtu.dk), at the Technical University of Denmark.

A web-based discussion forum (https://foros.isciii.es/viewforum.php?f=7) is also available for participants in the GMI PT 2016, allowing for individual sign-up and discussion with other PT-participants and the PT organizers in relation to issues relating to the analysis for the present PT. Appendix 4 of the PT protocol (see http://www.globalmicrobialidentifier.org/Workgroups/About-the-GMI-Proficiency-Test-2016) presents detailed information on the PT discussion forum. We strongly encourage the use of this forum as both a resource among participants but also as a platform to ask questions of the organizers – which other participants might also be interested in hearing the answer.

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

GMI is a global, visionary taskforce of scientists and other stakeholders who share an aim of applying novel genomic technologies and informatics tools to improve global patient diagnostics, surveillance and research, by developing needs- and end-user-based data exchange and analysis tools for characterization of all microbial organisms and microbial communities.

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In addition, the GMI PT 2016 is supported by the GenomeTrakr Network and Microbiologics®.

*	1.	Institute	name /	Organization	name
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* 2. Department name

* 3. Name of person responsible for the handling of the PT-material



* 4. Dates in relation to the handling of the PT-material (date for upload of sequence data)

	DD	MM	YYYY
Date PT-material received		/	1
Date processing the bacterial cultures started		/	/
Date processing the bacterial cultures completed		/	/
Date processing the DNA started		/	/
Date processing the DNA completed (upload of sequence data)		/	/

* 5. 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
- We did not receive bacterial cultures for this PT
- Other

If other, please define

* 6. Storage conditions of the DNA in the time between reception and processing: (please select one answer)

-80°C		
─ -20°C		
─ 4°C		
Room temperat	ure	
No storage time)	
Other		
If other, please define	9	

GMI Proficiency Testing 2016 - bacterial cultures and DNA

BACTERIAL CULTURES received

* 7. How were the bacterial cultures cultivated [as a decimal separator, please use full stop (.)]:

7.1 - Type of agar media/liquid broth:	
7.2 - Incubation time (hours):	
7.3 - Incubation temperature (°C):	

* 8. For the Gram-negative bacterial cultures; DNA extraction procedure (enter 'NA' if not relevant):

8.1 - If manual extraction; kit used, full name:

8.2 - If manual extraction; catalogue number of kit:

8.3 - If manual extraction, modifications to kit protocol:

8.4 - If automatic extraction; robot used:

8.5 - If automatic extraction; specific protocol:

8.6 - If automatic extraction; modifications to protocol:

* 9. For the Gram-positive bacterial cultures; DNA extraction procedure (enter 'NA' if not relevant):

9.1 - If manual extraction; kit used, full name:

9.2 - If manual extraction; catalogue number of kit:

9.3 - If manual extraction, modifications to kit protocol:

9.4 - If automatic extraction; robot used:

9.5 - If automatic extraction; specific protocol:

9.6 - If automatic extraction; modifications to protocol:

10. For bacterial cultures, DNA concentration (ng/µl) prior to library preparation was measured on (please select one answer)

Qubit
Nanodrop
ONA concentration not measured
Other
If other, please specify:

* 11. Measure of DNA concentration (ng/µl) [as a decimal separator, please use full stop (.)]

11.1 GMI16-001-BACT (Campylobacter)	
11.2 GMI16-002-BACT (Campylobacter)	
11.3 GMI16-003-BACT (Listeria)	
11.4 GMI16-004-BACT (Listeria)	
11.5 GMI16-005-BACT (Klebsiella)	
11.6 GMI16-006-BACT (Klebsiella)	

12. Total DNA amount (microgram) [as a decimal separator, please use full stop (.)]

12.1 GMI16-001-BACT (Campylobacter)	
12.2 GMI16-002-BACT (Campylobacter)	
12.3 GMI16-003-BACT (Listeria)	
12.4 GMI16-004-BACT (Listeria)	
12.5 GMI16-005-BACT (Klebsiella)	
12.6 GMI16-006-BACT (Klebsiella)	
	L

13. For bacterial cultures, DNA quality (e.g. RIN, 260/280 ratio and/or 260/230 ratio) prior to library preparation was measured on (please select one answer)

Bioanalyser
Nanodrop
DNA quality not measured
Other
If other, please specify:

14. Measure of DNA quality (e.g. RIN or 260/280 ratio) [as a decimal separator, please use full stop (.)]

14.1 GMI16-001-BACT (Campylobacter)	
14.2 GMI16-002-BACT (Campylobacter)	
14.3 GMI16-003-BACT (Listeria)	
14.4 GMI16-004-BACT (Listeria)	
14.5 GMI16-005-BACT (Klebsiella)	
14.6 GMI16-006-BACT (Klebsiella)	

15. If relevant; measure of DNA quality (260/230 ratio) [as a decimal separator, please use full stop (.)]

15.1 GMI16-001-BACT (Campylobacter)	
15.2 GMI16-002-BACT (Campylobacter)	
15.3 GMI16-003-BACT (Listeria)	
15.4 GMI16-004-BACT (Listeria)	
15.5 GMI16-005-BACT (Klebsiella)	
15.6 GMI16-006-BACT (Klebsiella)	

GMI Proficiency Testing 2016 - bacterial cultures and DNA

DNA received

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

If other, please specify:
Other
ONA concentration not measured
Nanodrop
Qubit

17. Measure of DNA concentration (ng/µl) [as a decimal separator, please use full stop (.)]

17.1 GMI16-001-DNA (Campylobacter)	
17.2 GMI16-002-DNA (Campylobacter)	
17.3 GMI16-003-DNA (Listeria)	
17.4 GMI16-004-DNA (Listeria)	
17.5 GMI16-005-DNA (Klebsiella)	
17.6 GMI16-006-DNA (Klebsiella)	

18. Total DNA amount (microgram) [as a decimal separator, please use full stop (.)]

18.1 GMI16-001-DNA (Campylobacter)	
18.2 GMI16-002-DNA (Campylobacter)	
18.3 GMI16-003-DNA (Listeria)	
18.4 GMI16-004-DNA (<i>Listeria</i>)	
18.5 GMI16-005-DNA (Klebsiella)	
18.6 GMI16-006-DNA (Klebsiella)	

19. For the DNA received, DNA quality (e.g. RIN, 260/280 ratio and/or 260/230 ratio) prior to library preparation was measured on (please select one answer)

Bioanalyser
Nanodrop
DNA quality not measured
Other
If other, please specify:

20. Measure of DNA quality (e.g. RIN or 260/280 ratio) [as a decimal separator, please use full stop (.)]

20.1 GMI16-001-DNA (Campylobacter)	
20.2 GMI16-002-DNA (Campylobacter)	
20.3 GMI16-003-DNA (Listeria)	
20.4 GMI16-004-DNA (Listeria)	
20.5 GMI16-005-DNA (Klebsiella)	
20.6 GMI16-006-DNA (Klebsiella)	

21. If relevant; measure of DNA quality (260/230 ratio) [as a decimal separator, please use full stop (.)]

21.1 GMI16-001-DNA (Campylobacter)	
21.2 GMI16-002-DNA (Campylobacter)	
21.3 GMI16-003-DNA (Listeria)	
21.4 GMI16-004-DNA (Listeria)	
21.5 GMI16-005-DNA (Klebsiella)	
21.6 GMI16-006-DNA (Klebsiella)	

22. Did you perform quality check to verify the quality of the DNA on a gel (see description in the protocol of this optional check)

	Yes	No
22.1 GMI16-001-DNA (Campylobacter)	\bigcirc	\bigcirc
22.2 GMI16-002-DNA (Campylobacter)	\bigcirc	\bigcirc
22.3 GMI16-003-DNA <i>(Listeria)</i>	\bigcirc	\bigcirc
22.4 GMI16-004-DNA <i>(Listeria)</i>	\bigcirc	\bigcirc
22.5 GMI16-005-DNA (Klebsiella)	\bigcirc	\bigcirc
22.6 GMI16-006-DNA (Klebsiella)	\bigcirc	\bigcirc

GMI Proficiency Testing 2016 - bacterial cultures and DNA

SEQUENCING

23. What 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

For commercial kits; full kit name:	
For commercial kits; catalogue number:	
For commercial kits; lot number:	
For noncommercial kits; citation for the protocol:	
For noncommercial kits; summary of the protocol:	
Deviations from the kit or cited protocol	

- * 24. Please indicate the sequencing platform you used in the proficiency test (please select one answer)
 - O Ion Torrent PGM
 - O 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
 - Genome Analyzer lix
 - 🔵 MiSeq
 - MiSeq Dx
 - MiSeq FGx
 - ABI SOLID
 - other

If other, please specify

25. Sequencing details #1

(please select one answer)

Single-end

Paired-end

Not relevant

26. Sequencing details #2:

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

* 27. 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 sequencer 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 sequencer), please indicate with 'yes' to this question]

\bigcirc	Yes	
\bigcirc	No	

If trimmed, which tool was applied (in the text box below, please insert name and URL/link (if possible))

28. 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? in the text box below, please insert name and URL (e.g. Velvet, https://www.ebi.ac.uk/~zerbino/velvet/, open access)

Assembly tool:

GMI Proficiency Testing 2016 - bacterial cultures and DNA

ANALYSIS of sequences

29. 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

Other (please specify)

30. 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

31. 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

32. For the DNA from the received bacterial culture, if MLST-analysis was performed based on the sequence analysis, which MLST-type does the isolate belong to?

32.1 GMI16-001-BACT (Campylobacter)	
32.2 GMI16-002-BACT (Campylobacter)	
32.3 GMI16-003-BACT (Listeria)	
32.4 GMI16-004-BACT (Listeria)	
32.5 GMI16-005-BACT (Klebsiella)	
32.6 GMI16-006-BACT (Klebsiella)	

33. For the DNA from the received bacterial culture, if MLST-analysis was performed based on the sequence analysis, which alleles characterize the isolate?

33.1 GMI16-001-BACT (Campylobacter)	
33.2 GMI16-002-BACT (Campylobacter)	
33.3 GMI16-003-BACT (<i>Listeria</i>)	
33.4 GMI16-004-BACT (<i>Listeria</i>)	
33.5 GMI16-005-BACT <i>(Klebsiella)</i>	
33.6 GMI16-006-BACT (<i>Klebsiella</i>)	

34. For the DNA from the received bacterial culture, if analysis for antimicrobial resistance genes was performed based on the sequence analysis, which antimicrobial resistance genes does the isolate harbour (please list the genes according to the following order of antimicrobial classes: Aminocyclitols, aminoglycosides, ß-lactams, fluoroquinolones, glycopeptides, lincosamides, macrolides, oxazolidones, phenicols, pleuromutilins, polypeptide antibiotics, quinolones, streptogramins, sulfonamides, tetracyclines, trimethoprim, other)?

34.1 GMI16-001-BACT (Campylobacter)	
34.2 GMI16-002-BACT (Campylobacter)	
34.3 GMI16-003-BACT (Listeria)	
34.4 GMI16-004-BACT (Listeria)	
34.5 GMI16-005-BACT (Klebsiella)	
34.6 GMI16-006-BACT (Klebsiella)	

35. For the received DNA, if MLST-analysis was performed based on the sequence analysis, which MLST-type does the isolate belong to?

35.1 GMI16-001-DNA (Campylobacter)	
35.2 GMI16-002-DNA (Campylobacter)	
35.3 GMI16-003-DNA (Listeria)	
35.4 GMI16-004-DNA (Listeria)	
35.5 GMI16-005-DNA (Klebsiella)	
35.6 GMI16-006-DNA (Klebsiella)	

36. For the received DNA, if MLST-analysis was performed based on the sequence analysis, which alleles characterize the isolate?

36.1 GMI16-001-DNA (Campylobacter)	
36.2 GMI16-002-DNA (Campylobacter)	
36.3 GMI16-003-DNA (Listeria)	
36.4 GMI16-004-DNA (Listeria)	
36.5 GMI16-005-DNA (Klebsiella)	
36.6 GMI16-006-DNA (Klebsiella)	

37. For the received DNA, if analysis for antimicrobial resistance genes was performed based on the sequence analysis, which antimicrobial resistance genes does the isolate harbour (please list the genes according to the following order of antimicrobial classes: Aminocyclitols, aminoglycosides, ß-lactams, fluoroquinolones, glycopeptides, lincosamides, macrolides, oxazolidones, phenicols, pleuromutilins, polypeptide antibiotics, quinolones, streptogramins, sulfonamides, tetracyclines, trimethoprim, other)?

37.1 GMI16-001-DNA (Campylobacter)	
37.2 GMI16-002-DNA (Campylobacter)	
37.3 GMI16-003-DNA (Listeria)	
37.4 GMI16-004-DNA (Listeria)	
37.5 GMI16-005-DNA (Klebsiella)	
37.6 GMI16-006-DNA (Klebsiella)	

38. For the detection of the Multi Locus Sequence Type, which tool did you apply? in the text box below, please insert name and URL (e.g. MLST 1.7 (MultiLocus Sequence Typing), http://cge.cbs.dtu.dk/services/MLST/, open access)

Tool for detection of MLST:

39. For the detection of the resistance genes harboured in the seqences, which tool did you apply? in the text box below, please insert name and URL (e.g. ResFinder, http://cge.cbs.dtu.dk/services/ResFinder/, open access)

Tool for detection of resistance genes:

GMI Proficiency Testing 2016 - bacterial cultures and DNA

SUBMITTED datafiles

 * 40. The obtained non-assembled sequence data have been uploaded for bacterial cultures and DNA following the description of batch-upload in the PT-protocol, for

	Yes	No
Campylobacter	\bigcirc	\bigcirc
Listeria	\bigcirc	\bigcirc
Klebsiella	\bigcirc	\bigcirc
Comments:		

Info:

For both bacterial cultures and DNA, the submitted sequence data (fastq-files) will be evaluated according to the following specific quality markers, e.g. read length (bp), N50 (bp), total number of contigs and total length of sequence (bp) including percentage of reference genome covered. In addition, the PT-organizers will assemble the submitted reads and compare these assemblies 1) towards the relevant closed genome to assess the sequence error rate and coverage of the scaffold and 2) between the obtained sequences for both the bacterial cultures and DNA.

GMI Proficiency Testing 2016 - FASTQ dataset

Introduction

This survey seeks to capture info in relation to the fastq data set component of the GMI Proficiency test (PT) 2016.

If you have any questions or feedback for the submission of data via this survey, please contact the PT Coordinator, Susanne Karlsmose Pedersen (suska@food.dtu.dk), at the Technical University of Denmark.

A web-based discussion forum (https://foros.isciii.es/viewforum.php?f=7) is also available for participants in the GMI PT 2016, allowing for individual sign-up and discussion with other PT-participants and the PT organizers in relation to issues relating to the analysis for the present PT. Appendix 4 of the PT protocol (see http://www.globalmicrobialidentifier.org/Workgroups/About-the-GMI-Proficiency-Test-2016) presents detailed information on the PT discussion forum. We strongly encourage the use of this forum as both a resource among participants but also as a platform to ask questions of the organizers – which other participants might also be interested in hearing the answer.

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

GMI is a global, visionary taskforce of scientists and other stakeholders who share an aim of applying novel genomic technologies and informatics tools to improve global patient diagnostics, surveillance and research, by developing needs- and end-user-based data exchange and analysis tools for characterization of all microbial organisms and microbial communities.

The GMI proficiency test 2016 is supported by COMPARE, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 643476.

In addition, the GMI PT 2016 is supported by the GenomeTrakr Network and Microbiologics®.

* 1. Institute name / Organization name

* 2. Department name

*	3.	Name(s)	of	person(s)	responsible	for the	analysis
---	----	---------	----	-----------	-------------	---------	----------

GMI Proficiency Testing 2016 - FASTQ dataset

FASTQ data set

4. Were reads quality filtered before conducting the analysis? (please select one answer)

Yes

No

5. If reads were quality filtered, please provide the name of the program

6. For variant detection, which of the following did you use:

(please select one answer)

- A reference based approach
- De novo assemblies
- A combination of both
- * 7. If you perform *de novo* assemblies, which tool did you apply (please insert name and URL; e.g. Velvet, https://www.ebi.ac.uk/~zerbino/velvet/)?

(Enter 'NA' if you do not do de novo assemblies)

* 8. If you use a reference-based approach, which tools do you use for mapping and variant detection (please insert name and URL; e.g. Bowtie2, http://bowtie-bio.sourceforge.net/bowtie2/index.shtml; VarScan, http://varscan.sourceforge.net)?

(Enter 'NA' if you do not use a reference-based approach)

* 9. What kind of methodology for phylogeny construction did you apply?

SNPs

Methodology other than SNPs

If methodology other than SNPs (please specify):

If applying SNPs, go to question 10, If not applying SNPs, go to question 12

10. Which quality criteria did you use for SNP calling? (e.g. % of mapped reads and minimum coverage to define variant).

10.1 - Campylobacter	
10.2 - Listeria	
10.3 - Klebsiella	

11. Which criteria did you use for SNPs filtering:

11.1 - Filter SNPs with excess coverage (i.e. repetitive regions):

11.2 - Did you filter SNPs occurring in a cluster (a.k.a. pruning) (indicate 'yes' or 'no'):

11.3 - Which definition of the cluster did you use (i.e. \geq 3 SNPs in 1000 base pairs (bp):

11.4 - Other, please specify:

12. Which program did you use to build your tree (e.g., MEGA, MrBayes, PAUP*, GARLI, RAxML, etc)?

13. Which algorithm did you use to build your tree (e.g., Neighbor-joining, UPGMA, Bayesian, maximumlikelihood, etc)?

Please upload to the ftp-site your DNA sequence matrix as a fasta alignment file (see description in the PT-protocol)

14. If you do assemblies, do you calculate the number of contigs (please select one answer)

С	Yes
С) No
\subset	We don't perform assemblies
15	. If you do assemblies, do you filter out contigs below a certain size (please select one answer)
\bigcirc) Yes
\bigcirc) No
\subset	We don't perform assemblies
lf y	res, indicate minimum size
4.0	
	. If you do assemblies, do you calculate N50 (please select one answer)
() Yes
() No
(We don't perform assemblies
17	. If you perform assemblies, do you calculate the size of the chromosome (please select one answer)
() Yes
\subset) No
\subset	We don't perform assemblies
C	
18	. Do you calculate coverage as a quality metric? (please select one answer)
C	Yes
C) No
)	
	. Did you check for contamination and/or verify the species? If so, which tool did you apply?
	the text box below, please insert name and URL (e.g. KmerFinder 1.2, p://cge.cbs.dtu.dk/services/KmerFinder)

(Enter 'NA' if you do not check for contamination and/or verify the species)

20. If you did check, could you verify the species?	

We did not attempt to verify species
Yes, for all Campylobacter
Yes, for all <i>Listeria</i>
Yes, for all <i>Klebsiella</i>
No, for some Campylobacter
No, for some <i>Listeria</i>
No, for some <i>Klebsiella</i>
If no, please indicate why

GMI Proficiency Testing 2016 - FASTQ dataset

SUBMITTED datafiles

Please carefully follow the instructions regarding the naming of submitted files and the samples that should be included in them! Thank you.

* 21. The following files have been submitted to the ftp-site:

	Campylobacter dataset	<i>Listeria</i> dataset	Klebsiella dataset
A fasta formatted DNA sequence matrix that was used for clustering (e.g., a fasta file extension)	\$	\$	\$
A newick formatted file with the clusters themselves (.tre file extension) (the format can be obtained through the R package APE or using FigTree's "Export Trees" option)		\$	\$
vcf (variant call format) files for each sample if a reference based approach was used and such files were produced	¢ t	\$	\$
Comments:			

The number and identity of samples in each uploaded file should match exactly those that were included in the original data (i.e., 15, 20, 17 sequences of *Campylobacter jejuni*, *Listeria monocytogenes* and *Klebsiella pneumoniae*, respectively). Within each file the samples should be named as the prefix within the original fastq file and the file should be named

GMILabID_Taxon.appropriateFileExtenation (e.g., GMI01_cj.fasta). See the protocol for specific instructions and additional information on the samples to be included and the naming of them.

GMI Proficiency Test Forum guide

Global Microbial Identifier



Content

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The purpose of the *GMI Profiency Test* forum is to facilitate the exchange of information and experiences among GMI PT participants.

Sign up to forum platform

GMI Profiency Test forum is hosted by the *Instituto de Salud Carlos III* (*Spanish National Health Institute Carlos III*). The first step is to register and create an account, which is described below.

Go to the GMI PT forum web page (*https://foros.isciii.es/viewforum.php?f=7*) and press the *Register* button.

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The board requires	es you to be registered and logged in to view this forum.	
Username:		
Password:	I forgot my password Remember me Hide my online status this session	
REGISTER	Login	
	ust be registered. Registering takes only a few moments but gives you increased capabilities. The board administrator may also grant additional permissions to registered use sure you are familiar with our terms of use and related policies. Please ensure you read any forum rules as you navigate around the board. sy policy	rs. Before
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Read carefully forum terms and press *I agree to these terms* to accept agreement.

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By accessing "" (hereinafter "we", "us", "our", ", "thtp://foros.iscili.es"), you agree to be legally bound by the following terms. If you do not agree to be legally bound by all of access and/or use " We may change these at any time and we'll do our utmost in informing you, though it would be prudent to review this regularly yourself as your conting you agree to be legally bound by these terms as they are updated and/or amended. Our forums are powered by phpBB (hereinafter "they", "them", "their", "phpBB software", "www.phpbb.com", "phpBB Limited", "phpBB Teams") which is a bulletin board so Public License v2" (hereinafter "GPL") and can be downloaded from www.phpbb.com. The phpBB software only facilitates internet based discussions; phpBB Limited is n disallow as permissible content and/or conduct. For further information about phpBB, please see: https://www.phpbb.com/. You agree not to post any abusive, obscene, vulgar, slanderous, hateful, threatening, sexually-orientated or any other material that may violate any laws be it of your court	nued usage of " after changes mean lution released under the "GNU General ot responsible for what we allow and/or
International Law. Doing so may lead to you being immediately and permanently banned, with notification of your Internet Service Provider if deemed required by us. The aid in enforcing these conditions. You agree that " have the right to remove, edit, move or close any topic at any time should we see th. As a user you agree to any inform in a database. While this information will not be disclosed to any third party without your consent, neither " nor phpBB shall be held responsible for any hacking attempt th compromised.	IP address of all posts are recorded to ation you have entered to being stored
I agree to these terms	
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Enter your username (with a length between 3 and 20 characters), email address and set up a password (must be between 8 and 15 characters long, must contain letters in mixed case and numbers). Additionally you could change your time zone. Enter confirmation code and press *Submit*.

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Quick links () FAQ	() Login
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- Registration	
Username: Length must be between 3 characters and 20 characters. Email address: Password Password must be between 8 characters and 15 characters long, must contain letters in mixed case and must contain letters in mixed case and must contain numbers. Confirm password: Language: My timezone:	British English
CONFIRMATION OF REGISTRATI	DN
	ions the board requires you to enter a confirmation code. The code is displayed in the image you should see below. If you are visually impaired or cannot otherwise read this code
	Reset Submit
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A welcome email will be sent to your email address. Click on *Return to the index page* and log in using your selected username and password.

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Information	
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Request to join GMI PT forum group

Joining as a member of GMI PT forum requires to be accepted by *Micro_Bio_GMI* group. Membership is restricted to *GMI Proficiency Test* participants. This section details the procedure to request that you be added to that group.

Click on a link located at the upper right corner of the screen that is labeled with your username. Click on *User Control Panel* and select *Usergroups* tab.

Quick links (2) FAC		🔔 Notifications [0] 🖂 Private messages [0] • xurxobm
Board Index		All User Control Pan
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Overview Profile	Board preferences Private messages Usergroups Friends & Foes	
	MEMBERSHIPS Registered users This is a special group, special groups are managed by the board administr	select
	PENDING MEMBERSHIPS	SELECT

Clik on *Micro_Bio_GMI* radio button. Select *Join selected* in *Select* dropdown list and press *Submit* button.

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Manage groups	placed in or allowed to join other group	s. Some groups may gi	e you additional permissions	to view content or increas	se your capabilities in othe	r areas.	SELECT
	Registered users This is a special group, special group	s are managed by the b	oard administrators.				O
	NON-MEMBERSHIPS						SELECT
	Micro_Bio_GMI This is an open group, members can	apply to join.					۲
						Select: Join selected	▼ Submit
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Confirm request to GMI PT forum group.

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E Quick links ② FAQ	🗘 Notifications [0] 🖂 Private messages [0] • xurxobm2 *
Request to join group	
Are you sure you want to request joining the selected group?	
Yes No	
♦ Board index	Contact us 🗎 Delete all board cookies • All times are UTC+02:00

Your request must be approved by GMI PT forum moderator. It should take not more than a few days. You will see a notification in the upper right corner of the screen when your request is approved.

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	ncy Test r: jbarrera	0 0	No posts	
New Topic * Search this forum Q				0 topics • Page 1 of 1
There are no topics or posts in this forum.		<u>19</u>		
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Introduce yourself to other GMI PT participants

The first time you log into the forum after gaining admission to the *Micro_Bio_GMI* group were accepted you should introduce yourself. This is not mandatory but is recommended to facilitate communication among all GMI PT participants.

Click on *General* category link (if you do not see *Global Microbial Identifier* forum categories, see Troubleshooting, page 12).

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Global Microbial Identifier Moderator: Jarrera					Mark subforums read
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There are no topics or posts in this forum.			_		
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Click on *Introductions* category link.

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Introduce yourset, your lab and your research interests, etc	0	0	No posts	
ftp site for upload / download B Issues about the site for downloading data and uploading results Moderator: Jaurea	0	0	No posts	
Collection of seq. parametres (survey Monkey) Buses about survey Moderator: Darrea	0	0	No posts	
Discussions / Conclussions General discussions and conclusions about methods, protocols, etc Moderator: Javrena	0	0	No posts	
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Write a post introducing yourself and your lab and press *Submit* to publish it.

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Introductions		
POST A NEW TOPIC		
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Hello everyone, My name is Jorge de la Barrera. I work on [I]CNM[/i] (I]National Center for Microbiology[/i] Bioinformatics Unit of ISCIII (I]Sp Institute Carlos III[/I]). This is a core service unit that provides bioinformatic analysis to all agencies belonging to ISCIII. I am f for microbiology for CNM researchers and reference labs. Cheers Jorge de la Barrera		
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Once post is published it can be read by all GMI PT participants within *Introductions* forum category.

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Hello to everyone Moderator: partera		
Post Reply e Search this topic Q, 🔯		1 post - Page 1 of 1
Hello to everyone Disy wwwist - 14 Apr2015, 11:22 Hello everyone, My name is Jorge de la Barrera. I work on CMM (National Center for Microbiology) Bioinformatics Unit of ISCIII (Span Carlos III). This is a core service unit that provides bioinformatic analysis to all agencies beionging to ISCIII. I am for microbiology for CNM researchers and reference tabs. Cheers	ish National Health Institute	www.def offic:2 Prined:11 Mar 2015, 15:55
Jorge de la Barrera		0
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Post a message on GMI PT forum

GMI PT forum is organized in categories to improve readability. When you want to write a message you should choose the right forum category to post on. This section details GMI PT forum categories.

Forum categories are organized in two branches: *General* and *Proficiency Test*. No messages can be posted at the main level.

FORUM			TOPICS	POSTS	LAST POST
	Global Microbial Identifier	General Moderator: jbarrera	1	1	Hello to everyone by xurxobm2 G April 14th, 2015, 11:47 am
	Global Microbial Identifier	Proficiency Test Moderator: jbarrera	0	0	No posts

Introduction posts, issues about ftp site or survey and discussions/conclusions must be posted in the suitable category under *General* branch.

General Moderator: jbarrera

			Mark subforums read
FORUM	TOPICS		
(B) Introductions Introduce yoursel, your lab and your research interests, etc Moderator janarea	0	0	No posts
Bits Issues about the site for downloading data and uploading results Moderator: participartindex	0	0	No posts
Collection of seq. parametres (survey Monkey) Issues about survey Moderator: parera	0	0	No posts
Biscussions / Conclussions General discussions and conclusions about methods, protocols, etc Moderator parera	0	0	No posts

Profiency Test branch encompass categories related to the PT itself.

Proficiency Test Moderator: jearera Mark subforums read Mark subf

<u>Wet lab</u> and whole genome sequencing issues must be posted in the suitable category under *DNA extraction / Purification / Library Preparation / WGS* category.

DNA extraction / Purification / Libray Preparation / WGS

			Mark subforums read
FORUM	TOPICS		
Bacterial, cultures Moderator: parrera	0	0	No posts
Urus Moderator: jbarrera	0	0	No posts

Dry lab issues must be posted in the suitable category under *Bioinformatics*.

Bioinformatics Moderator: jbarrera

			Mark subforums read
FORUM	TOPICS		LAST POST
Bactorial, Data sets	0	0	No posts
Wirus Moderator; jbarrera	0	0	No posts

Troubleshooting

Problem: You are not authorized to read this forum

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This message is shown if you have not registered.

Solution: Register as user following steps described in this guide.

Problem: Global Microbial Identifier forum categories are not shown

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