The EURL-AR WGS EQA:

DTU Genomic PT

Susanne Karlsmose Pedersen (suska@food.dtu.dk)
On behalf of the EURL-AR

# EU Reference Laboratory for AMR (EURL-AR)

- Funded by the EU Commission

NRL

Created with mapchart.net

SeqAfrica

- Fleming Fund Regional Grant
- Extending Whole Genome Sequencing (WGS) capacity for AMR surveillance across Africa
- Regional WGS Centres perform genomic surveillance of major bacterial pathogens and provide sequencing & data analysis services and training to the African region within a One Health framework
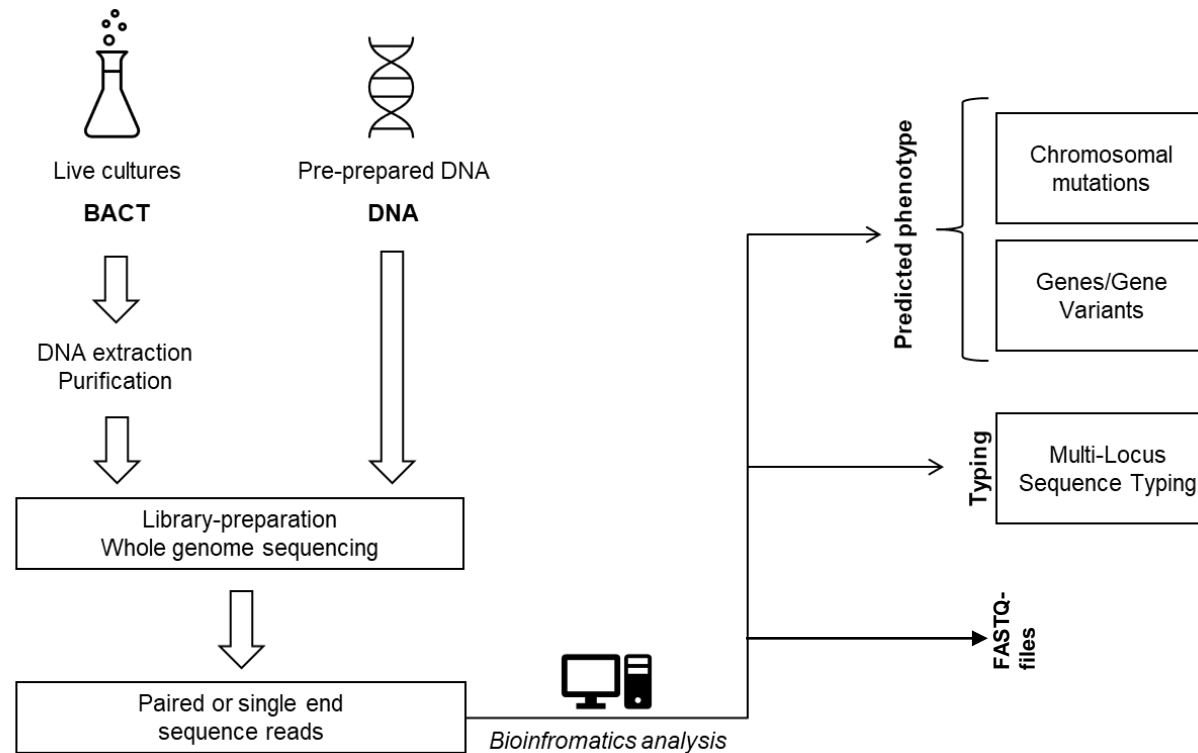

SeqAfrica

- Regional WGS Centres (2019-2023)
- Regional WGS Centres (2019-2025)
- New Regional WGS Centres (2023-2025)
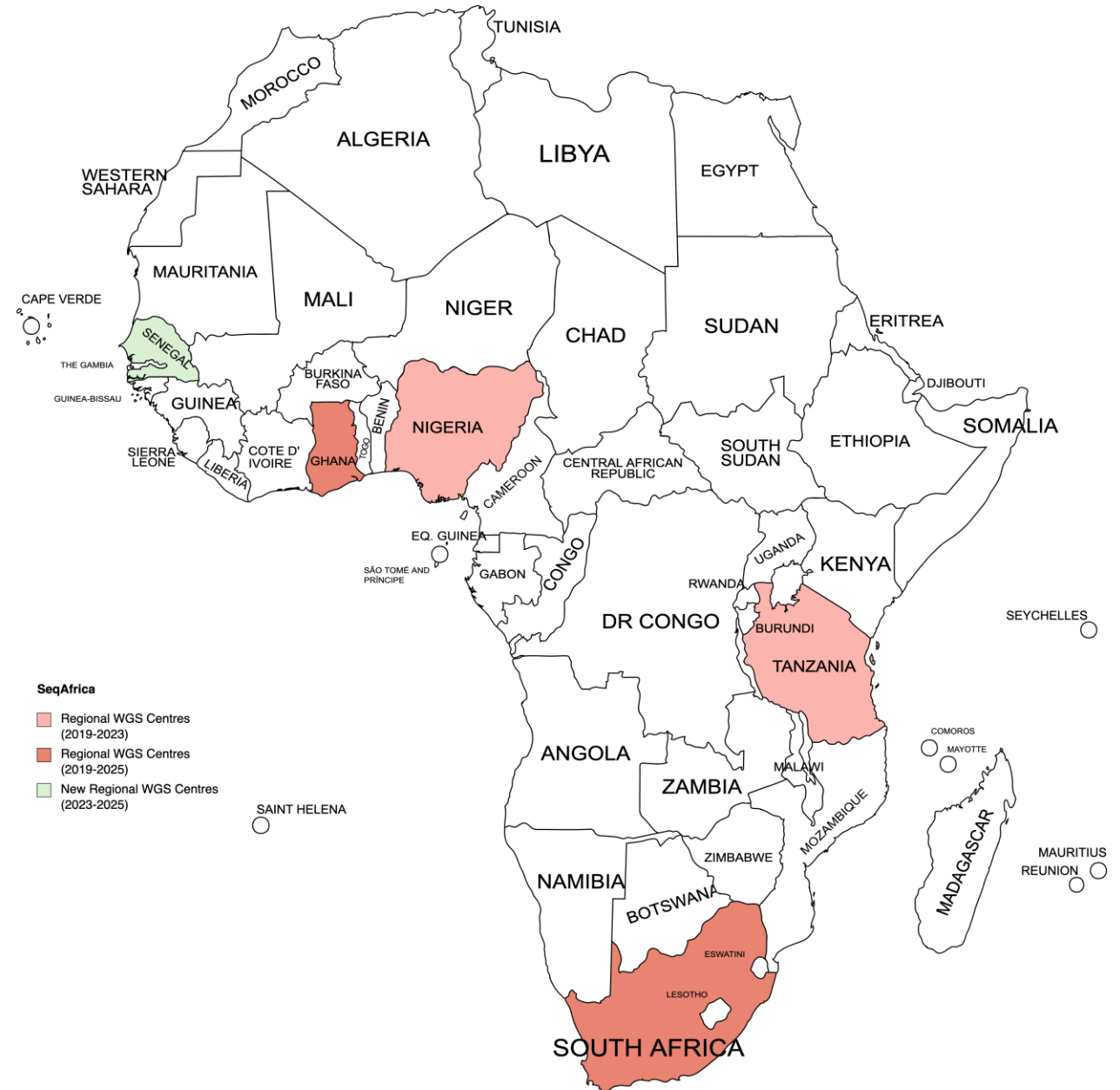
DTU Genomic PT – brief introduction

Each year, six strains included, two of each organism:

	2021	2022	2023	2024
E. coli	X	X	X	X
Salmonella	X		X	
Campylobacter coli/jejuni	X			X
Enterococcus faecium/faecalis		X		X
Staphylococcus aureus		X	X	

Genomic PT – setup

Schematic overview of the downstream processing of live culture samples (BACT) and pre-prepared DNA samples (DNA).
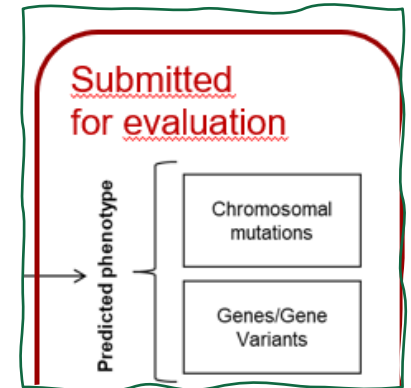
Genomic PT – setup
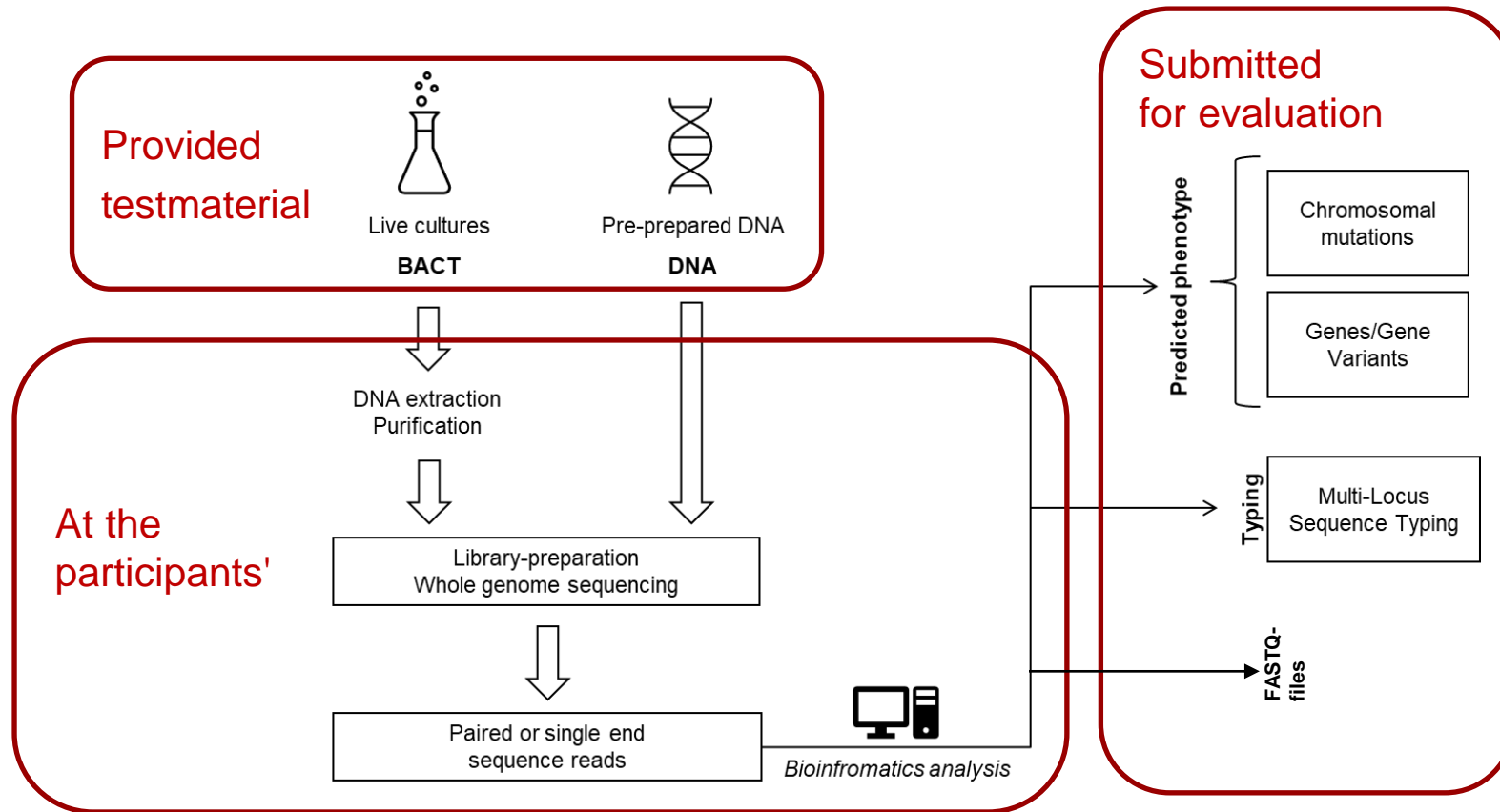
Schematic overview of the downstream processing of live culture samples (BACT) and pre-prepared DNA samples (DNA).
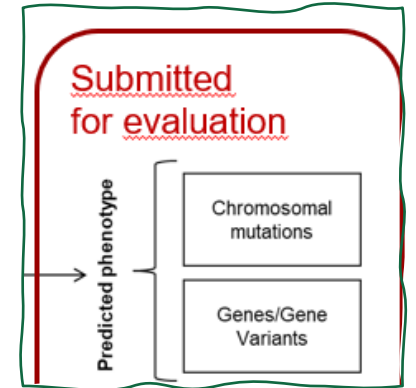
DTU Genomic PT – participants instructions

Table 2: Antimicrobial agents included in the current PT for *S. aureus*

Antimicrobial	Class
Cefoxitin	Beta-lactam
Chloramphenicol	Amphenicol
Ciprofloxacin	Quinolone
Clindamycin	Lincosamide
Erythromycin	Macrolide
Fusidate	Steroid antibacterial
Gentamicin	Aminoglycoside
Kanamycin	Aminoglycoside
Linezolid	Oxazolidinone
Mupirocin	Pseudomonic acid
Penicillin	Beta-lactam
Rifampin	Rifamycin
Streptomycin	Aminoglycoside
Sulfamethoxazole	Folate pathway antagonist
Tetracycline	Tetracycline
Tiamulin	Pleuromutilin
Trimethoprim	Folate pathway antagonist
Vancomycin	Glycopeptide

Submitted for evaluation

Predicted phenotype → Chromosomal mutations / Genes/Gene Variants

DTU Genomic PT – submission of results

Via a password-protected webtool

DTU Genomic PT – submission of results

Evaluation report

Submitted for evaluation
- Predicted phenotype → Chromosomal mutations / Genes/Gene Variants

Organism *Staphylococcus aureus*

Method

Question	Answer
1.Date processing of Staph	
2.Date processing of Staph	
3.Date processing of Staph	
4.Date processing of Staph	
5.How were the bacterial c	
5.1.Type of agar media/liqu	
5.2.Incubation time (hours)	
5.3.Incubation temperature	

Antimicrobial Resistance

Strain	Material	Class	Obtained gene and gene variant	Expected gene and gene variant	Score
GENOMIC22-001	GENOMIC22-001-DNA	Amphenicol	fexA	fexA	1
GENOMIC22-001	GENOMIC22-001-DNA	Beta-lactam	mecA	mecA	1
GENOMIC22-001	GENOMIC22-001-DNA	Beta-lactam	blaZ	blaZ	1
GENOMIC22-001	GENOMIC22-001-DNA	Folate pathway antagonist	dfrG	dfrG	1
GENOMIC22-001	GE				
GENOMIC22-001	GE				
GENOMIC22-001	GE				
GENOMIC22-001	GE				
GENOMIC22-001	GE				

Predicted phenotype

Strain	Material	Obtained predicted phenotype	Expected predicted phenotype	Score
GENOMIC22-001	GENOMIC22-001-DNA	Cefoxitin Chloramphenicol Clindamycin Linezolid Penicillin Streptomycin Tetracycline Tiamulin Trimethoprim	(Cefoxitin,Chloramphenicol,Clindamycin,Linezolid,Penicillin,Streptomycin,Tetracycline,Tiamulin,Trimethoprim)	1
GENOMIC22-001	GENOMIC22-001-BACT	Cefoxitin Chloramphenicol Clindamycin Linezolid Penicillin Streptomycin Tetracycline Tiamulin Trimethoprim	(Cefoxitin,Chloramphenicol,Clindamycin,Linezolid,Penicillin,Streptomycin,Tetracycline,Tiamulin,Trimethoprim)	1

DTU Genomic PT
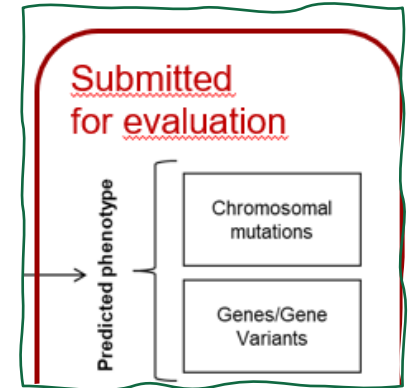
DTU Genomic Proficiency Test 2022

DTU Genomic Proficiency

A guide on the interpretation of the submitted data

EU Reference Laboratory for Antimicrobial Resis
National Food Institute
Technical University of Denmar

Author: Athina Andrea (atand@food.dt

Contact: Proficiency test coordinator Susanne Karlsmose Pe

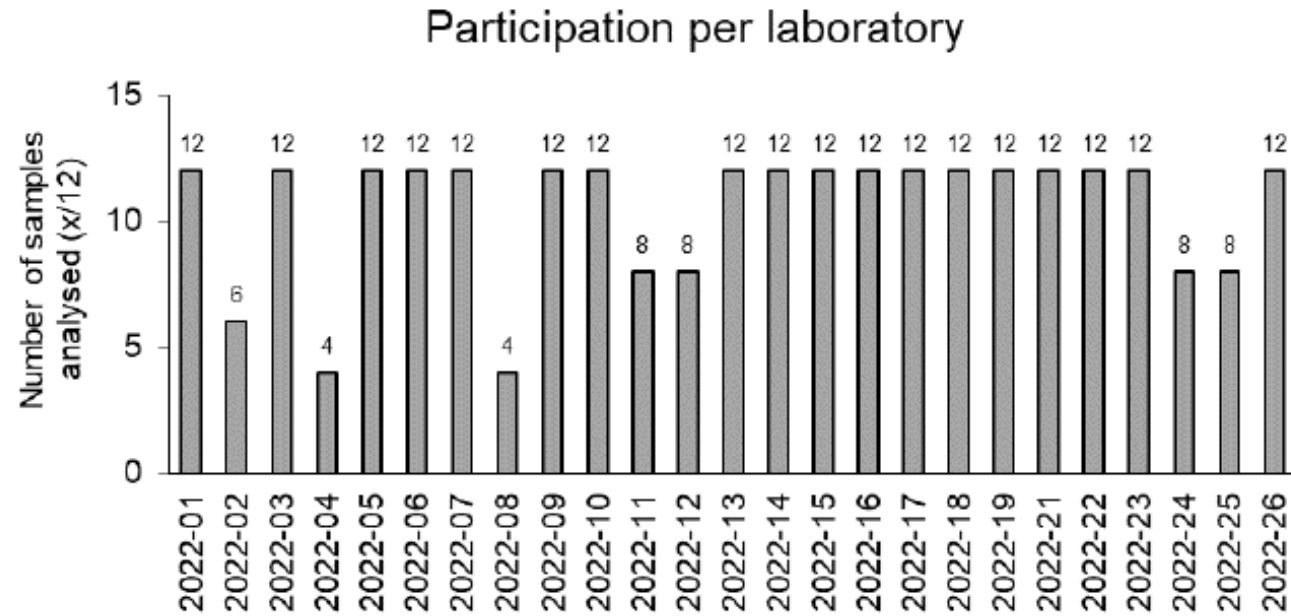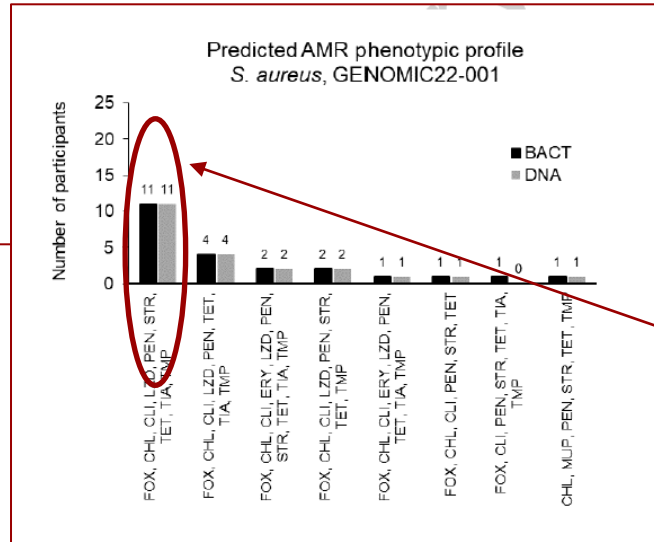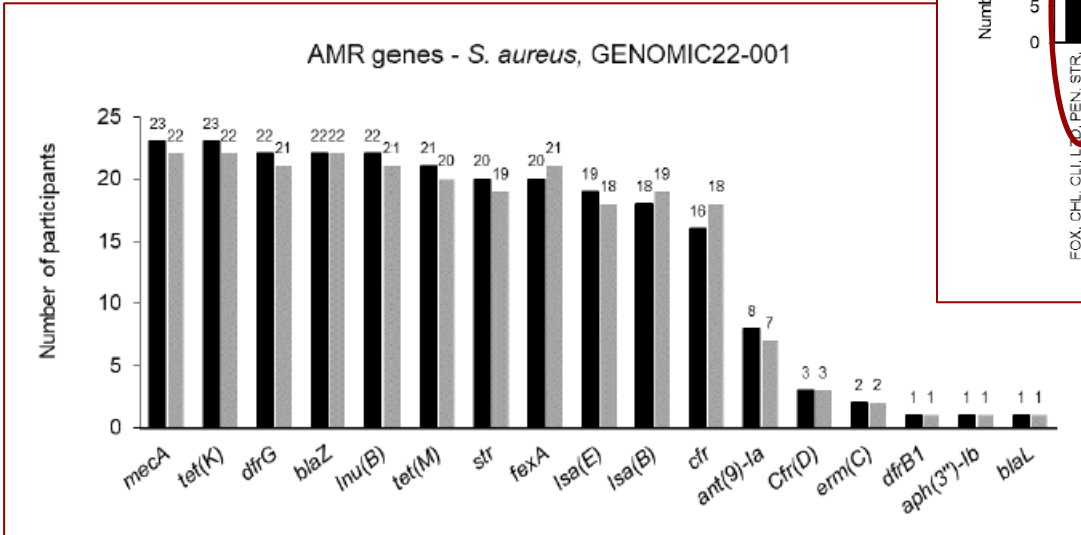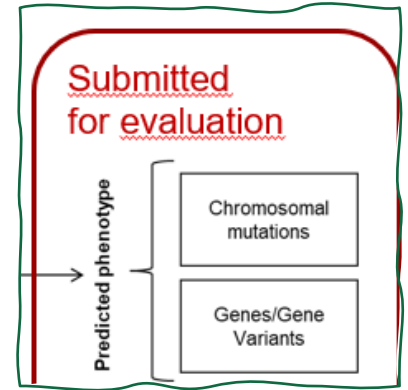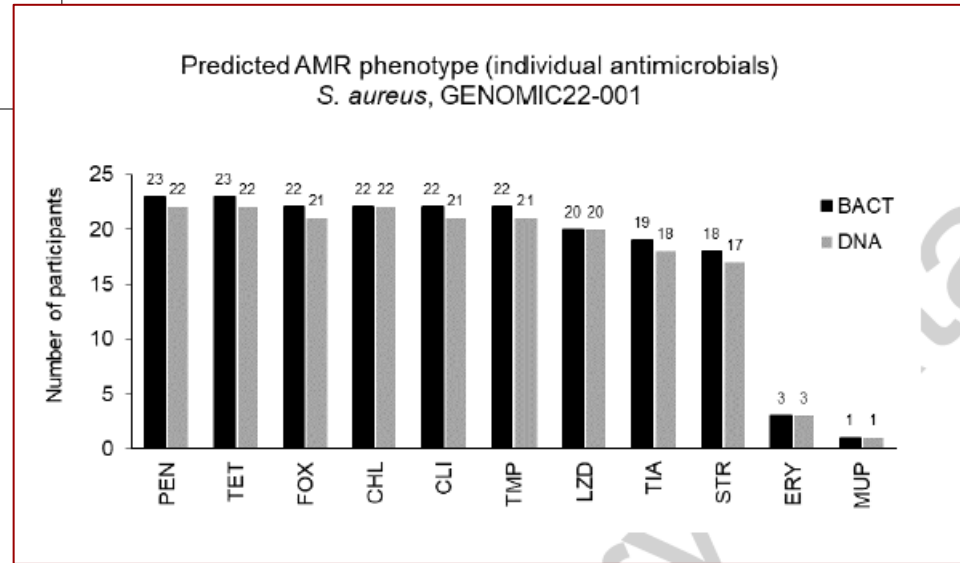Figure 2. Level of participation per laboratory in Genomic PT 2022

Data validation

Evaluation of sequence quality

Closed genome of all included test strains available

Some of the included QC parameters:
- # of reads
- # of reads mapped to reference chromosome / plasmid X / genome
- Coverage of reference chromosome / plasmid X / genome
- Depth of coverage of reference chromosome / plasmid X / genome
- Size of assembled genome
- Proportion of reads mapped to reference
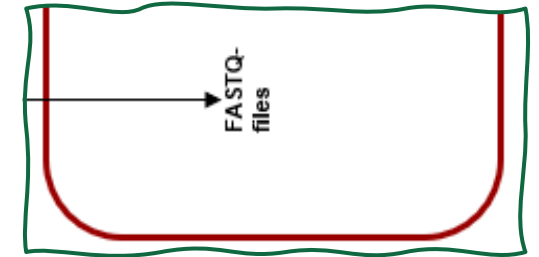- Total # of contigs
- # of contigs >200bp
- N50
- NG50
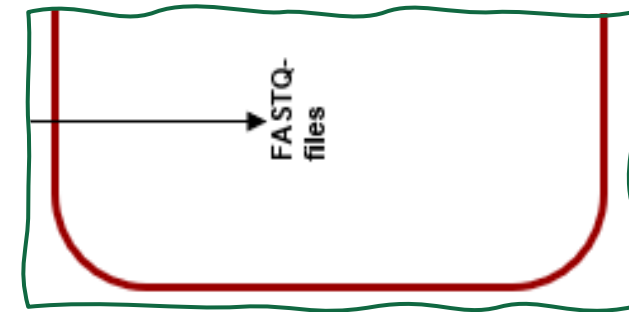
Reads mapping to reference

- Proportion of reads mapping to closed reference

- One boxplot per isolate

- Thresholds applied for scoring were minimal >80%, and preferably >90%

- Generally lower mapping proportion in GENOMIC22-005 (*Enterococcus faecium*)
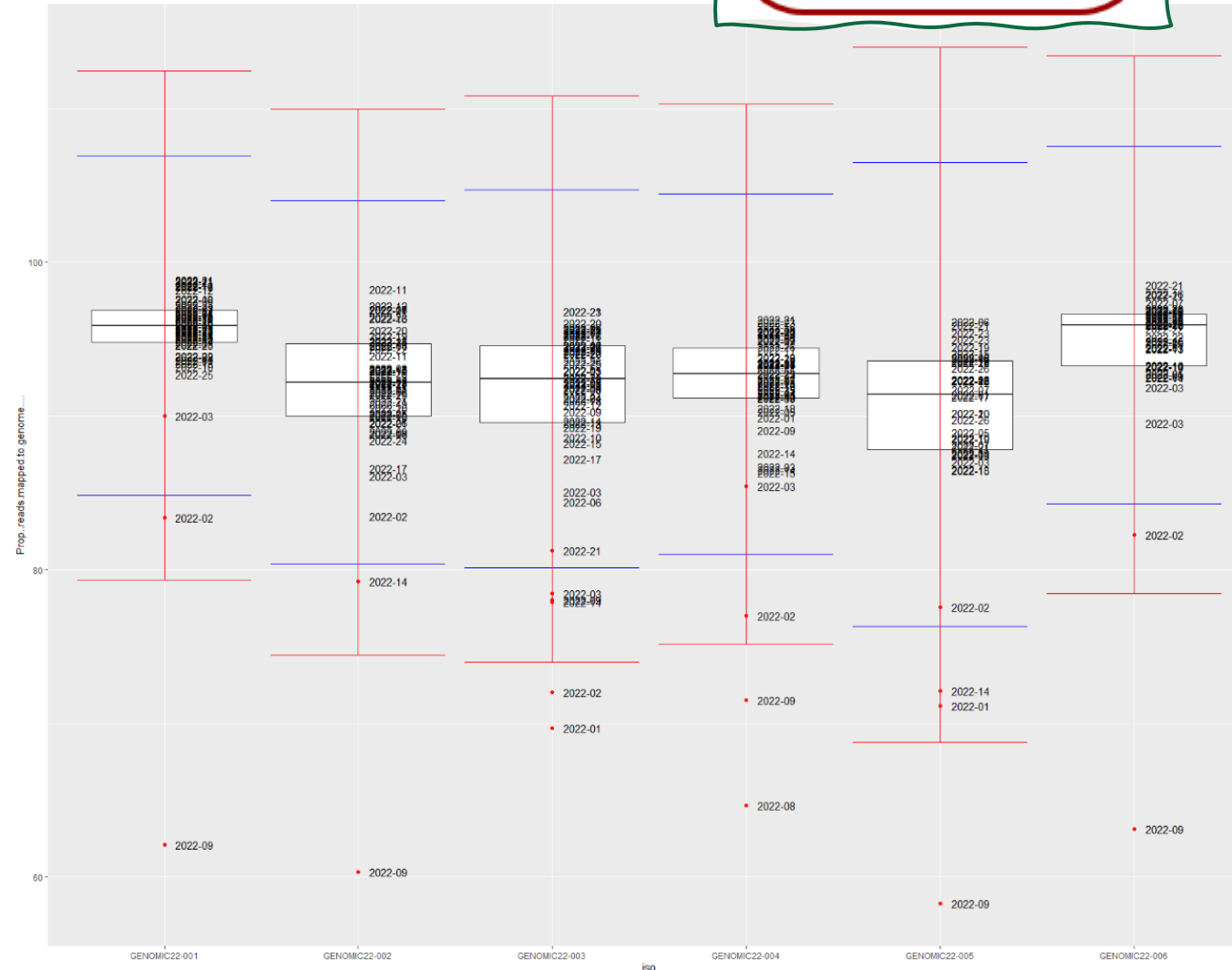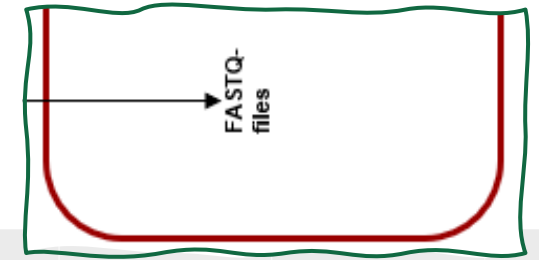
Bioinformatic analysis – N50

- N50 per isolate per DNA/BACT

- Blue whiskers:
  2 standard deviations from the mean

- Red whiskers:
  3 standard deviations from the mean

- Genomes larger than 110%
  or smaller than 90% of expected size
  have been excluded

- 3 genomes below 3 SD in isolates
  GENOMIC22-005 and -006
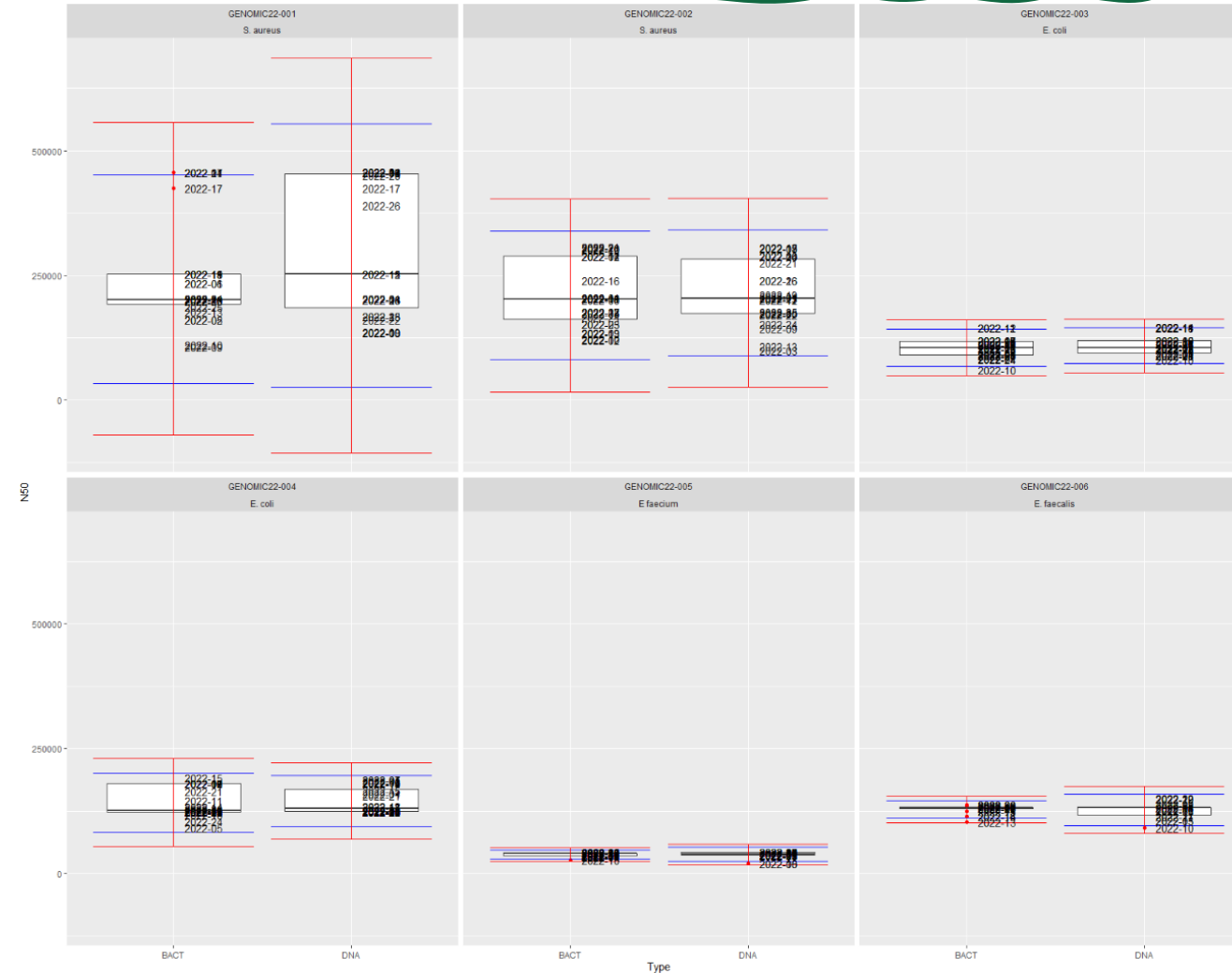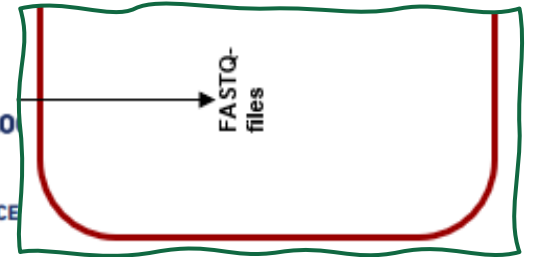
Results of the 2020 Genomic Proficiency Test for the network of European Union Reference Laboratory for Antimicrobial Resistance assessing whole-genome-sequencing capacities

Thea Kristensen[1,2,†], Lauge Holm Sørensen[3,*,†], Susanne Karlsmose Pedersen[3], Jacob Dyring Jensen[1], Hanne Mordhorst[1], Niamh Lacy-Roberts[3], Oksana Lukjancenko[4], Yan Luo[5], Maria Hoffmann[5] and Rene S. Hendriksen[3]

Abstract

The global surveillance and outbreak investigation of antimicrobial resistance (AMR) is amidst a paradigm shift from traditional biology to bioinformatics. This is due to developments in whole-genome-sequencing (WGS) technologies, bioinformatics tools, and reduced costs. The increased use of WGS is accompanied by challenges such as standardization, quality control (QC), and data sharing. Thus, there is global need for inter-laboratory WGS proficiency test (PT) schemes to evaluate laboratories' capacity to produce reliable genomic data. Here, we present the results of the first iteration of the Genomic PT (GPT) organized by the Global Capacity Building Group at the Technical University of Denmark in 2020. Participating laboratories sequenced two isolates and corresponding DNA of *Salmonella enterica*, *Escherichia coli* and *Campylobacter coli*, using WGS methodologies routinely employed at their laboratories. The participants' ability to obtain consistently good-quality WGS data was assessed based on several QC WGS metrics. A total of 21 laboratories from 21 European countries submitted WGS and meta[data]. delivered high-quality sequence data with only two laboratories identified as overall underperformi[ng]. number of contigs, were identified as good indicators for high-sequencing quality. We propose Q[C] than 20000 and 25000 for *Campylobacter coli* and *Escherichia coli*, respectively, and number of c[ontigs] 225, 265 and 100 for *Salmonella enterica*, *Escherichia coli* and *Campylobacter coli*, respectively. The G[PT] importance of systematic QC procedures, ensuring the submission of reliable WGS data for surveill[ance] gation to meet the requirements of the paradigm shift in methodology.

Upcoming, separately on:
- Sequence quality
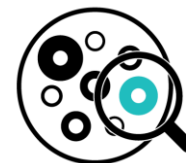- Sequence analysis (AMR components, MLST, serotype)

Lessons learnt

- Genes/gene variants – differences between tools/pipelines
    o e.g., CARD, ResFinder, AMRFinder+

- Predicted AMR profile
    o evaluated as a whole (one profile)
    o each antimicrobial individually

- Basis of scoring
    o Manual individual adjustments in the webtool
    o Lack of international guidelines for assessment of sequence quality

Acknowlegdements
- Athina Andrea, EURL-AR
- Lauge Sørensen, EURL-AR
- Rene S. Hendriksen, EURL-AR

Thanks for your attention