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# RING TRIALS QUALITY ASSURANCE Work group 4:

 $f(x+\Delta x) = \sum_{i=0}^{\infty} \frac{(\Delta x)^i}{i!} f^{(i)}(x)$ 

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#### WG Goal – What to do

Work group 4 will provide a plan to establish a proficiency testing based on whole genomes sequencing for the Global Microbial Identifier initiative

# Vision of WG 4

That all laboratories globally conducting NGS on bacteria and vira to the highest degree of quality in terms of detection of relevant genes, point mutations and phylogeny



# Parameters affecting sequence quality Technology and platforms

- DNA input requirements / Library preparation
- Comparison of read technologies / Read length assessments
- Platform specific errors no of Error-free reads rates
- Comparison of read technologies
- Sequence coverage depth and GC bias
- Assessment performance metrics at lower coverage

Comparison de novo genomes assembly; the long and short of it. (PLoS One. 2011 Apr 29; 6(4))

A tale of three next generation sequencing platforms: comparison of Ion Torrent, pacific Biosciences and Illumina Miseq sequencers. (BMC Genomics. 2012 Jul 24;13:341)

Performance comparison of whole genomes sequencing platforms. (Nat Biotechnol. 2011 Dec 18; 30(1): 78-82.)

Comparison of sequencing platforms for single nucleotide variant calls in human samples. (PLoS ONE 8(2): e55089.)

Evaluation of next generation sequencing platforms for population targeted sequencing studies. (Genome Biol. 2009; 10(3))

Biases in read coverage demonstrated by interlaboartory and interplatform comparison of 117 mRNA and genomes sequencing experiments. (BMC Bioinformatics. 2012 Apr 19; 13)

Performance comparison of benchtop high-throughput sequencing platforms. (Nat Biotechnol. 2012 May; 30(5): 434-9) DTU Food, Technical University of Denmark



# Parameters affecting sequence quality Data analysis

- Comparison of assembler algorithms
- Anomalies in assembly accuracy through eg.
  - N50
  - Coverage
  - Contig size
  - Etc.
- Ability to single nucleotide base variant calling
- Detection of indels and differences in size
- Technology-dependent variants Unmapped regions / missed variants
- Assessment of mappability to specific genes

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## **Mission of WG 4**

To provide a formal mechanism for inter-laboratory test performance to ensure harmonisation and standardisation in whole genome sequencing and data analysis, with the aim to produce comparable data



# WG objectives

- To initially organise a pilot proficiency test for the work group participants
- To secondly offer this to test to GMI members working with both bacteria and vira

## WG themes

- 1. Infrastructure: To build an infrastructure within the partners of GMI that has the capacity to undertake the facilitation of the proficiency testing.
- Reference material: To develop or provide the reference material and documents needed to initiate the proposed pilot proficiency test scheme. Disseminate reference material to enrolled laboratories. To adjust the reference material and documents as well as the analysis based on previous experiences.
- **3.** Genome analysis: To conduct the analysis of submitted genomes.
- 4. Virus experiences: To evaluate RNA purification methods / protocols and pilot sequencing on multiple platforms to initiate the proposed parallel viral pilot proficiency test scheme.
- Proficiency test: To execute fully operational proficiency test based on bacteria and vira to GMI members.



### Status of the WG

#### **Reference material / Genome analysis**

- Selection of suitable reference material (bacteria) for component 1; evaluate the quality of sequencing performed on different platforms / instruments
  - Public and animal health and food safety relevant bacteria
  - 3 isolates per trial eg. Salmonella, Campy, Vibrio
  - Blinded non-public closed genomes (DNA)
- Selection of a data set containing suitable reference genomes for component 2; assessing the quality of mapping the genomes to relevant genes using available bioinformatic pipelines

- Data set of eg. 20 assembled genomes (Salmonella)

- Analysis parameters; quality markers / target genes
  - Parameters to measure WGS quality



## Thank you for your attention



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