

EURL WORKING GROUP ON WHOLE GENOME SEQUENCING AND PULSENET INTERNATIONAL

EURL-*Campylobacter* workshop, 9/10-2018

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Objectives of the WG-NGS

- To promote the use of NGS across the EURL networks
- Build NGS capacity within the EU and among NRLs
- Ensure no overlap of activities between the WG and EFSA/ECDC

Participants of the WG

- EURL *E. coli* (WG coordinator)
- EURL *Listeria monocytogenes*
- EURL CPS (Coagulase Positive Staphylococci)
- EURL *Salmonella*
- EURL *Campylobacter*
- EURL *Parasites*
- EURL AR
- EURL Food borne viruses
- Observers: SANTE G4, EFSA, ECDC

- 8 tasks - each led by a task leader (different EURLs)
- All EURL members will contribute to ALL the tasks by bringing in the specific needs of their networks as well as their experience.

Proficiency Testing (EURL AR lead)

- Reports containing the main conclusions from the analyses performed in PTs on WGS carried out by EURLs
- Share experiences about and harmonize:
 - Methodology of PT organization
 - What data to be extracted from WGS and returned to the PT provider
 - How to analyse PT results by the EURLs

NGS laboratory procedures (EURL P lead)

- Evaluation of available DNA Extraction SOPs developed by existing projects (EU Compare, EFSA projects, ENGAGE and INNUENDO etc) to validate the performances and suitability for the NRLs network
- Work on reducing the time from culture to sequencing by testing different library preparation and sequencing kits

Bioinformatics tools (EURL VTEC lead)

- Development of guidance documents or reports on different bioinformatics tools for NGS data mining (with the exclusion of the cluster analyses)
- Defining criteria of selection of tools to support NRLs
- Develop, set up and (if possible) validate tools and pipelines for those NRLs that do not have/use proprietary software or other resources
- Some NRLs were contacted to elaborate on the tools used

WGS Cluster Analysis: (EURL Campy lead)

- Define criteria of selection of software (open source and commercial) to support NRLs in cluster analysis of WGS data
- Documents summarizing software, techniques, input demands, quality thresholds, pros/cons etc.
- These documents will assist training-task in producing teaching material
- We will contact NRLs that perform cluster analysis (from survey) for input

Bench marking (EURL Listeria lead)

- Development of guidance document defining how to benchmark analytical methods and pipelines

Trainings on WGS (EURL CPS lead)

- Definition of a common structure for training events (courses, eLearning, ad hoc training stages, organized jointly or by the single EURLs) to harmonize the trainings delivered to the NRLs.
- Practical training on WGS data production: DNA extraction, library preparation and operation of sequencing equipment
- WGS data analysis

Reference and confirmatory testing using WGS (EURL Salmonella Lead)

- Give suggestions for control strains for testing pipelines
- Development of guidance document for the use of and/or validation of the use of NGS for confirmatory testing and typing (e.g. use of WGS for *Salmonella* serotyping, testing for virulence genes of *E. coli*)

Summary of questionnaire on the support expected from the EURL on the application of Next Generation Sequencing technology, including WGS of bacterial/viral/parasites and metagenomics by the EU NRLs

Questionnaire sent out: 2018-03-23

Last date to reply: 2018-04-16

Questionnaire sent to: 35 MS NRLs and 7 non-MSs labs

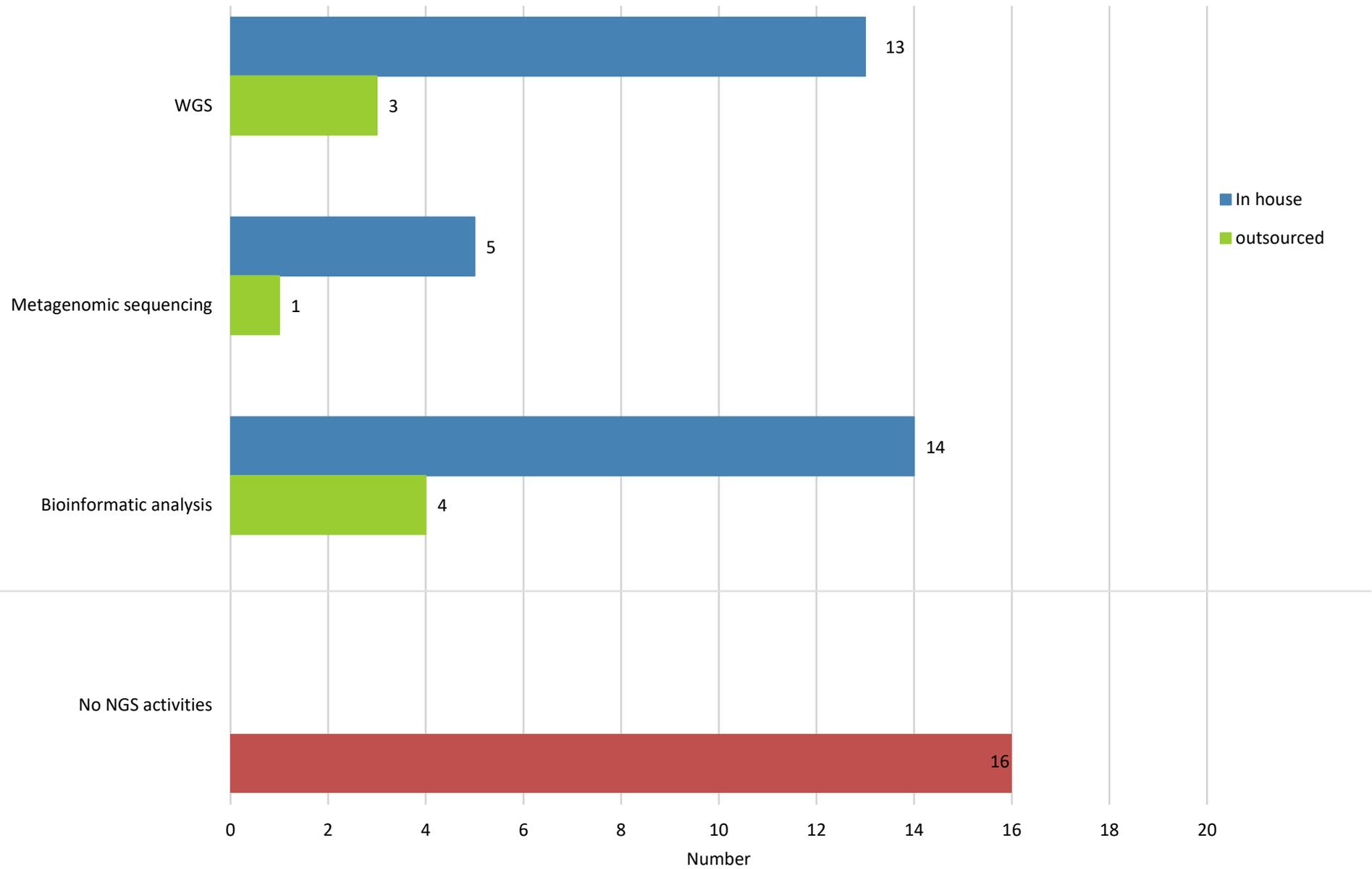
Number of participants in the questionnaire: 32

Same survey was sent to all EURL-networks.

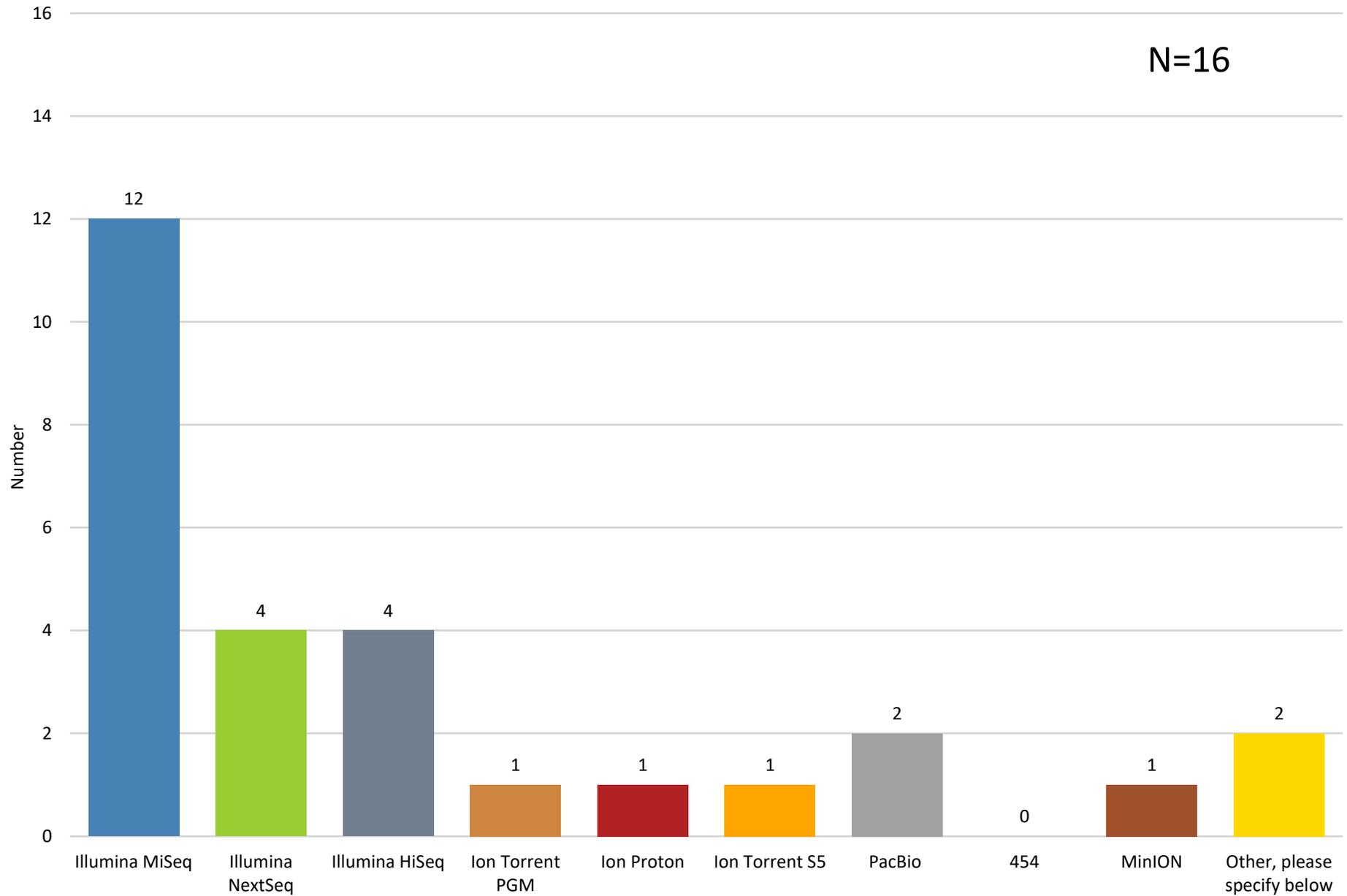
EFSA's survey was difficult to interpret with regards to which pathogen a certain method was used

The results of the whole survey will be made available later

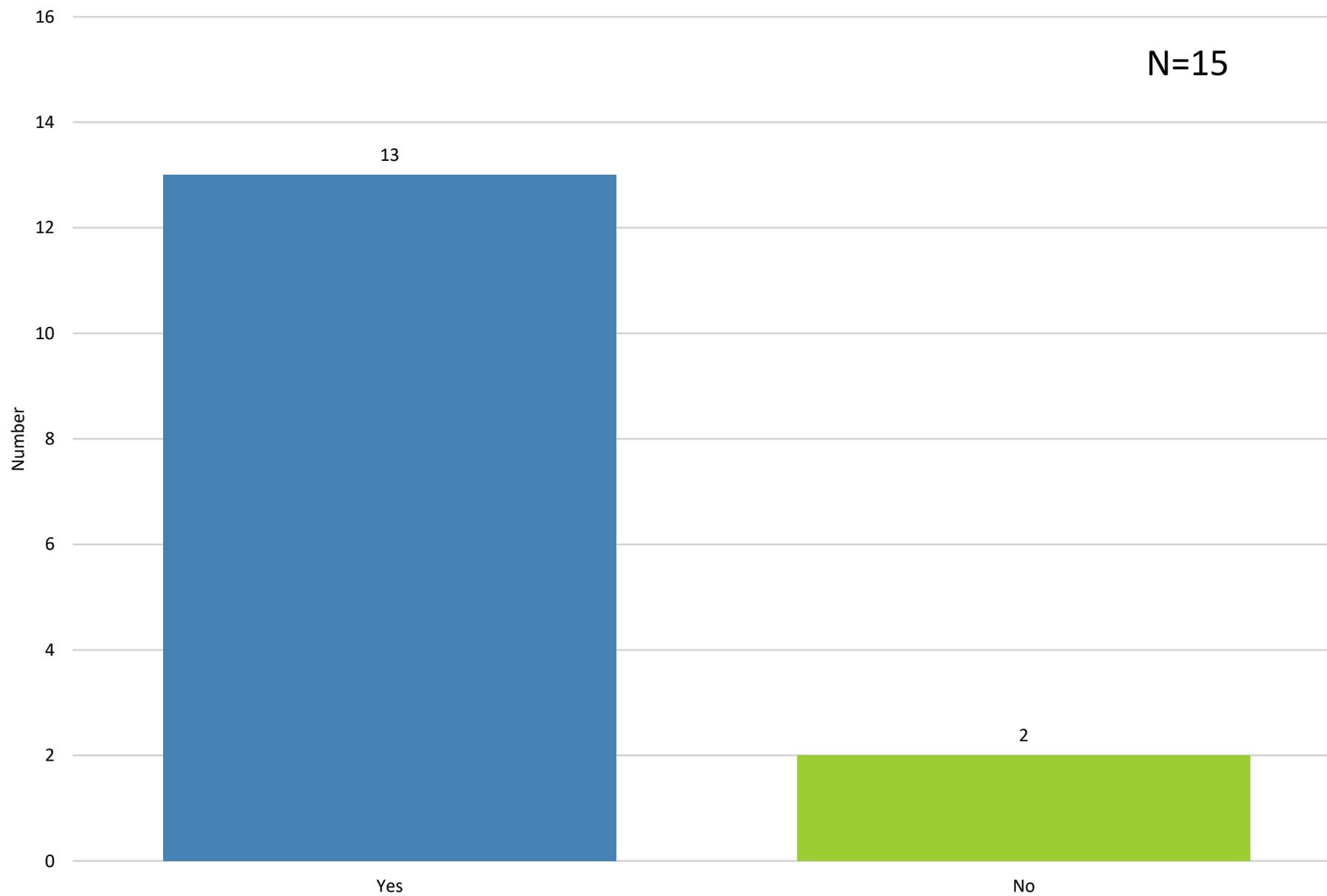
Q1. In your role of NRL, which of the following NGS activities do you perform? (multiple choice)



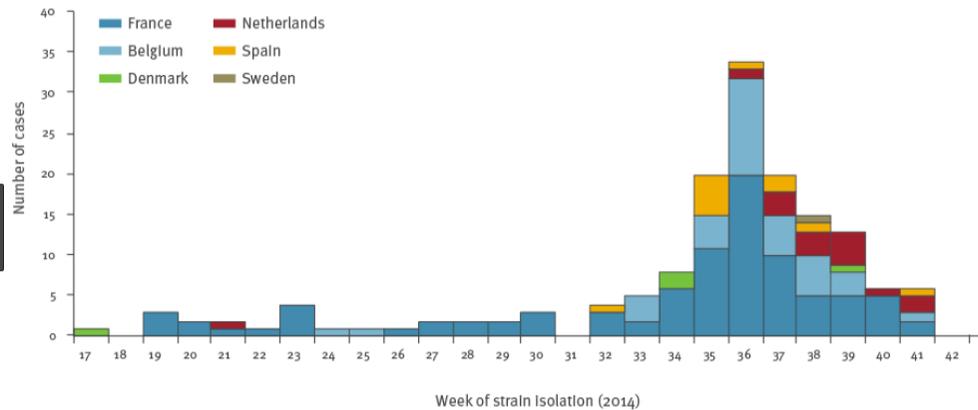
Q10. Please specify which NGS platforms you use and/or are used by the lab/company to which the activity is outsourced (multiple choice):



Q13. Do you perform cluster analysis? (single choice)



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For surveillance using WGS a standardised validated system is needed

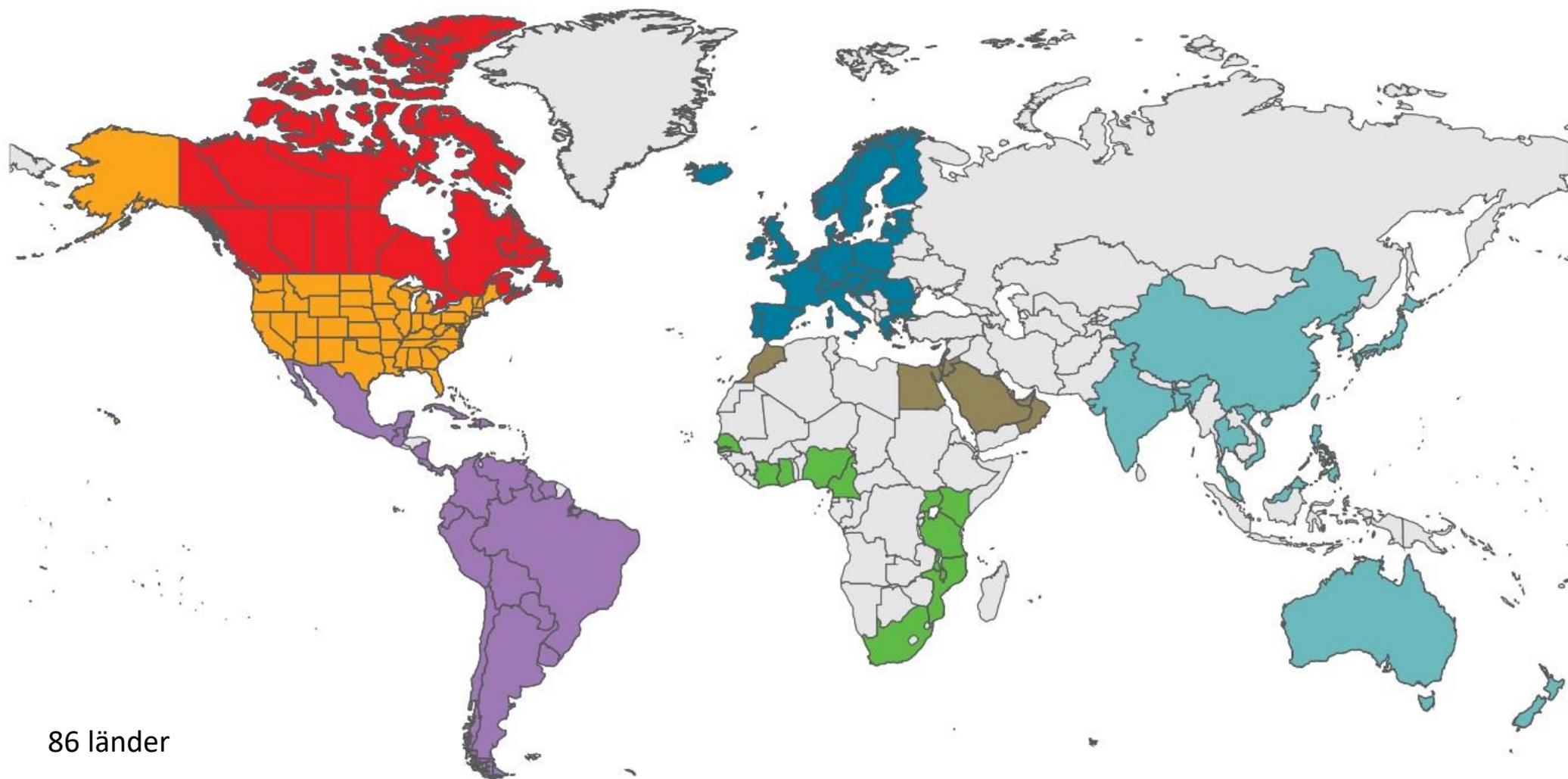
Institutes are now running different software with different parameters and using different online databases



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FIGURE 1

Map of PulseNet International participating countries, May 2017



86 länder

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REVIEW ARTICLES

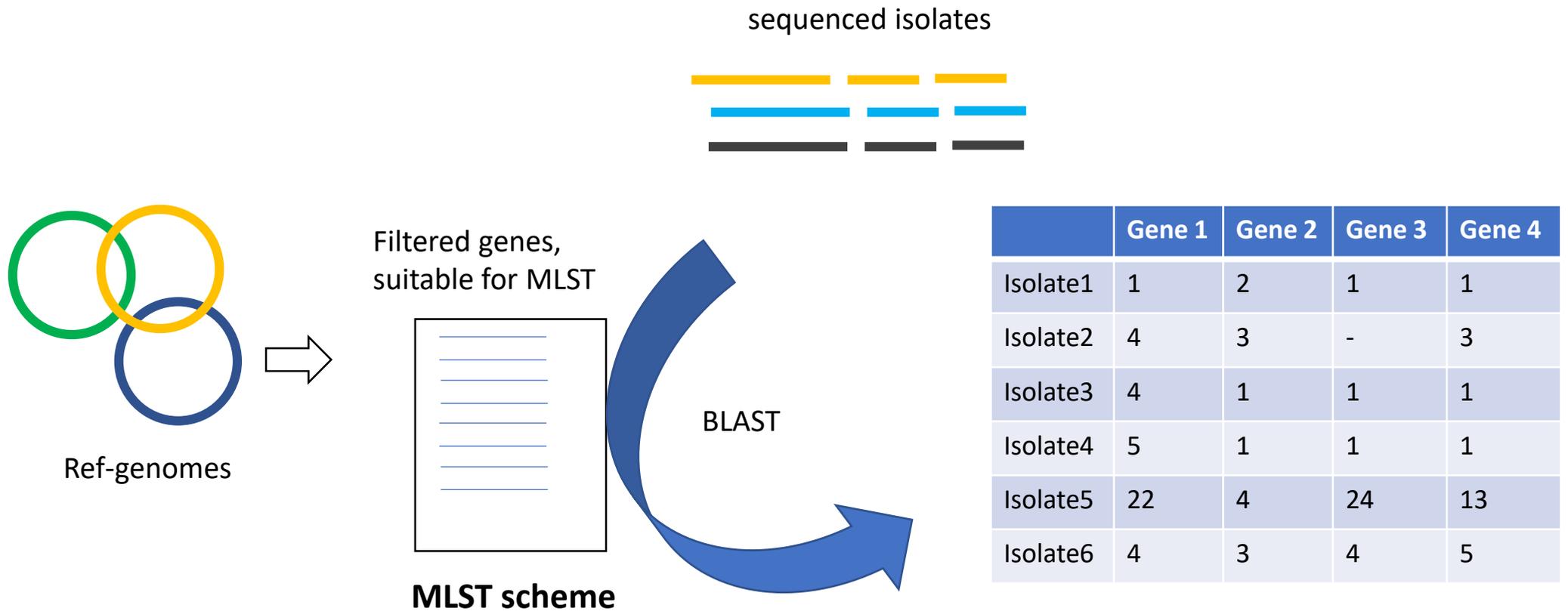
PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance

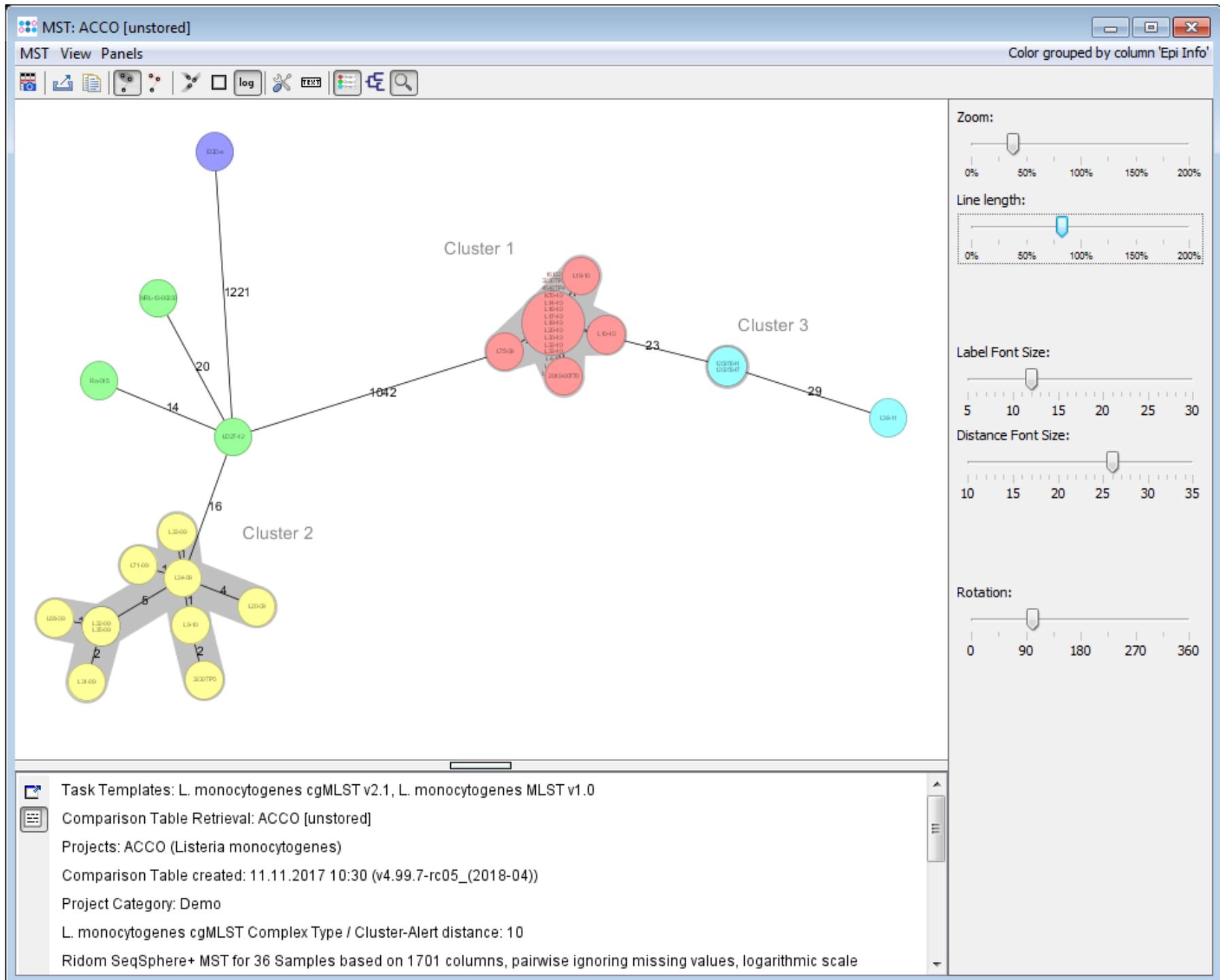
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12. The members of the FWD-NEXT Expert Panel are listed at the end of the article

MLST and WGS

- Extended MLST
- Core genome MLST, cgMLST
- Whole genome MLST, wgMLST
- Shared core genome MLST....





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PulseNet International meeting on global foodborne disease surveillance: Towards publicly available databases and tools

Goal: To reach consensus on getting wg/cgMLST databases and tools for analysis of WGS from bacterial foodborne pathogens out in public domain in order to facilitate international laboratory surveillance of foodborne disease including the prevalence of antimicrobial resistance traits in foodborne pathogens, and the detection and investigation of outbreaks.

Major outcomes:

Validating MLST schemes for each species.
-assembly
-allele calls
-epidemiological concordance



- One wg/cgMLST scheme for each organism.
- Allele and strain nomenclature should be in the public domain
- Depositing raw sequence data and metadata (provenance) should be optional
- Standardized strain nomenclature that is taxonomically informative should be used for wgMLST.
- Host(s) for publicly available databases and tools were not identified.

Salmonella
E. coli
Campylobacter
Listeria