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Bioinformatics Platform

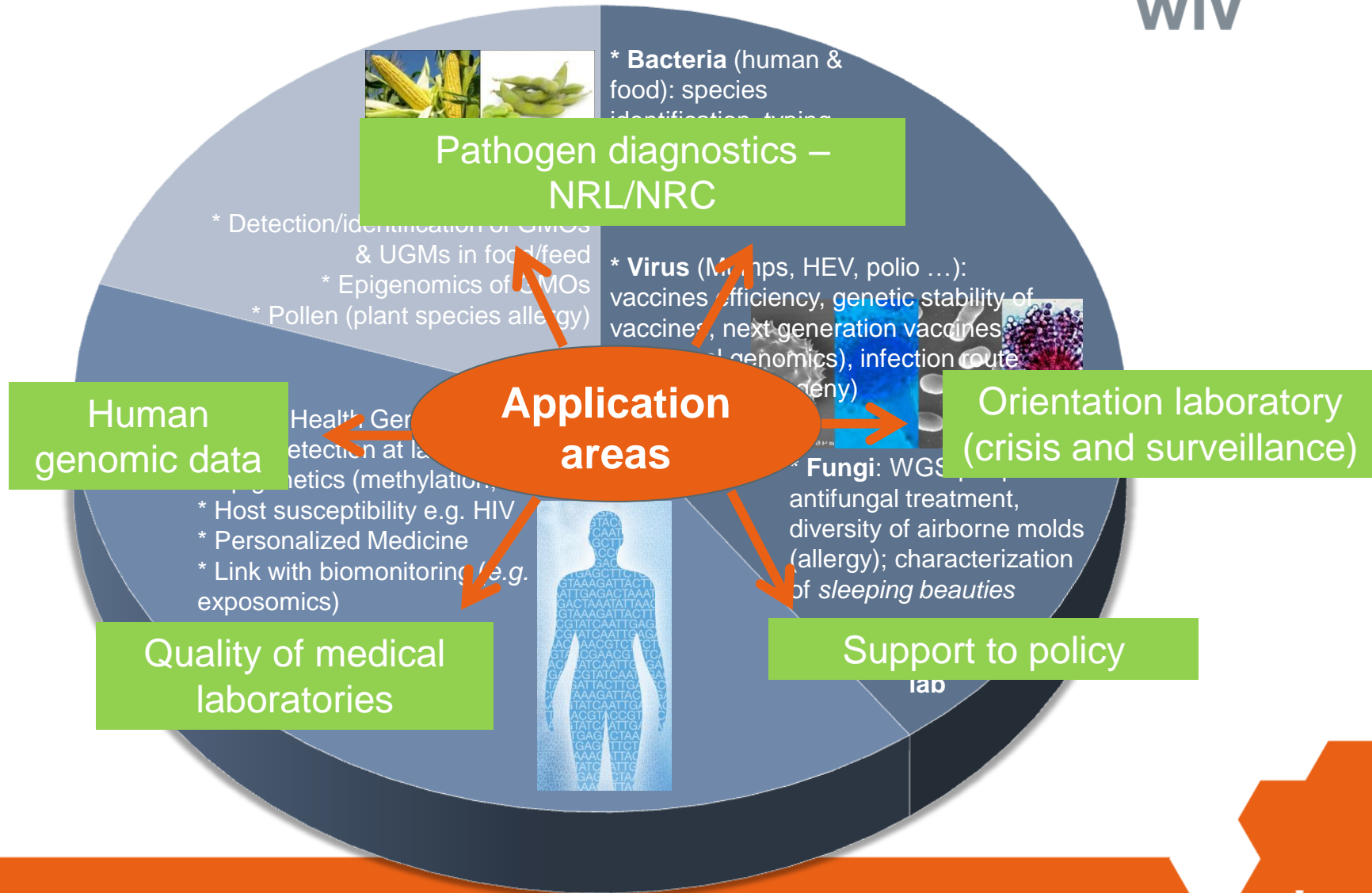
Platform Biotechnology and Molecular Biology

Department Expertise, Service Provision and Customer Relations

Collaboration between the EURL-VTEC and the Platform for Biotechnology and Molecular Biology (WIV-ISP, Belgium) for the development of a bioinformatics pipeline for routine analysis of whole genome sequencing data for typing of STEC/*E. coli* using Galaxy

12th Annual Workshop of the National Reference Laboratories for *E. coli* in the EU (12/10/2017-13/10/2017)

Use of NGS & bioinformatics @WIV-ISP?



The Next-Generation Sequencing revolution



NGS as a well established research tool...

- Universal method
- Single nucleotide resolution
- High-throughput, ultimate multiplex tool

...but many challenges remain regarding data analysis and interpretation for routine applications in a public health setting!

- Computational requirements
- Validation of standardized & optimized pipelines
- User-friendly access for non-experts
- Trade-off between quality and speed of analysis
- Traceability (databases, runs)

NGS & bioinformatics platform

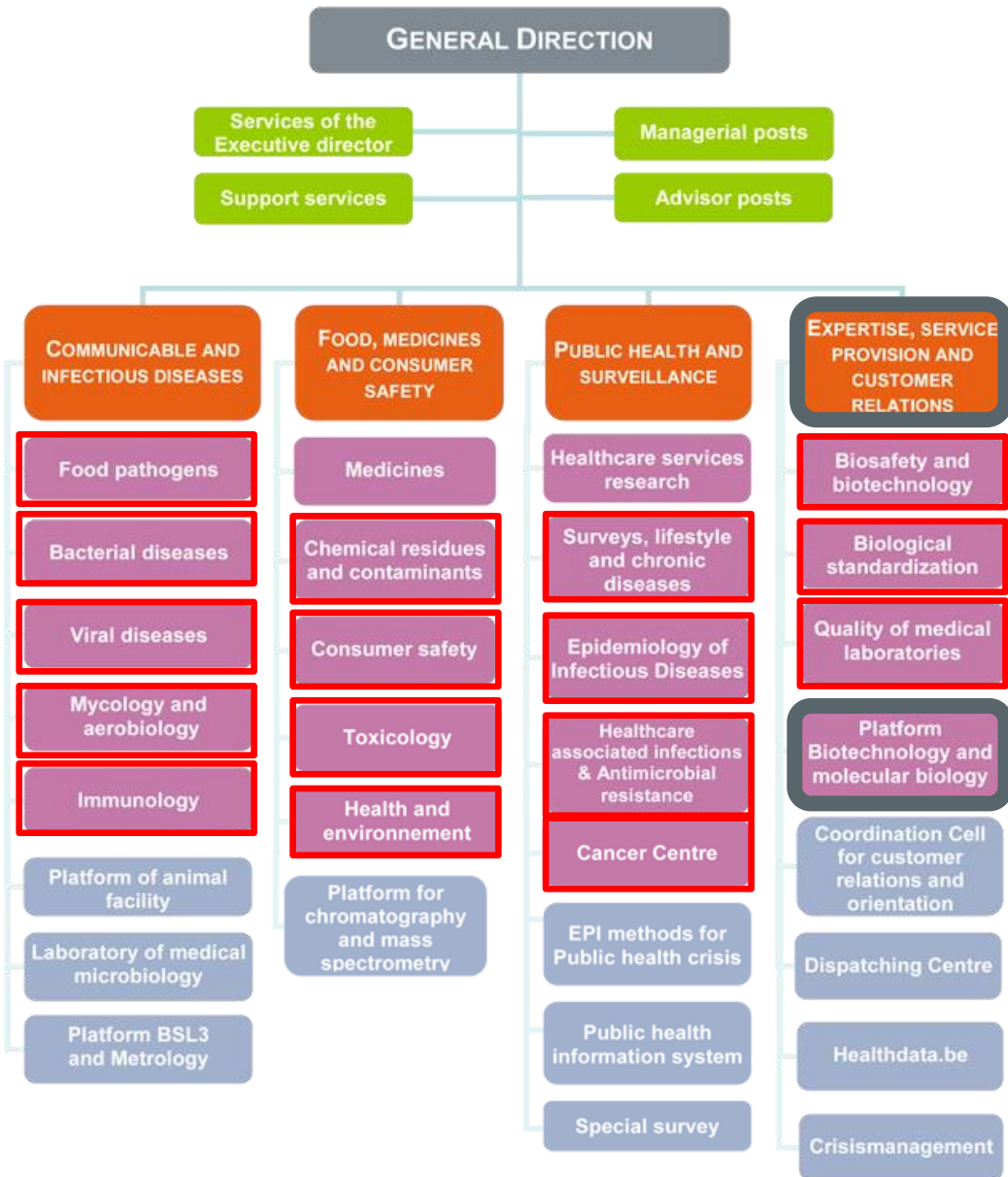


Mission: Utilizing NGS & bioinformatics

- for the diagnosis, surveillance, control and characterisation of potentially harmful organisms
- to promote public health genomics by the effective integration into clinical use and public health policy

Objectives: Develop and implement solutions and provide data acquisition and analysis tools to

- complement the WIV-ISP laboratories services
- integrate the knowledge of genomics into public health policy



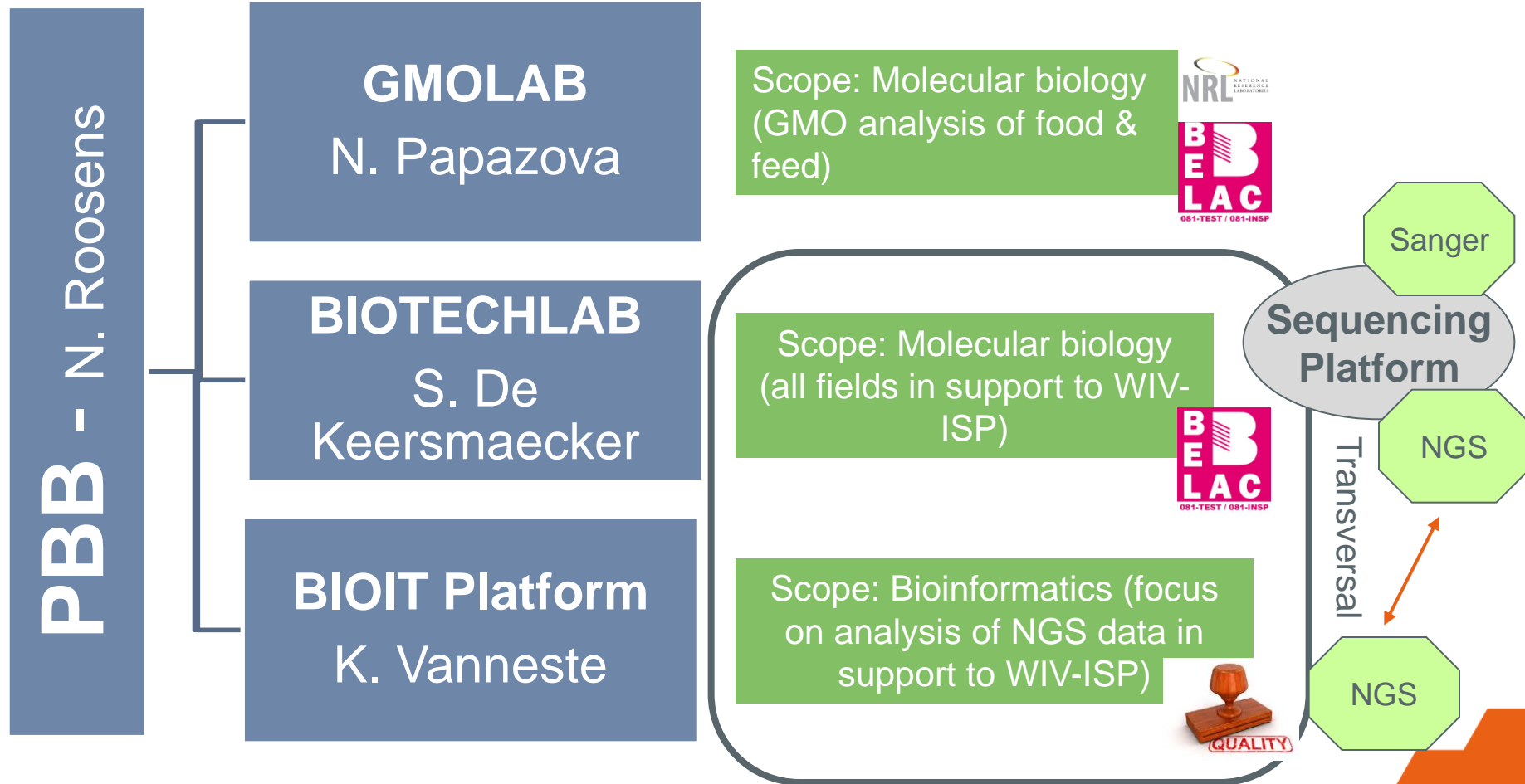
NGS & bioinformatics platform @WIV-ISP

Mission

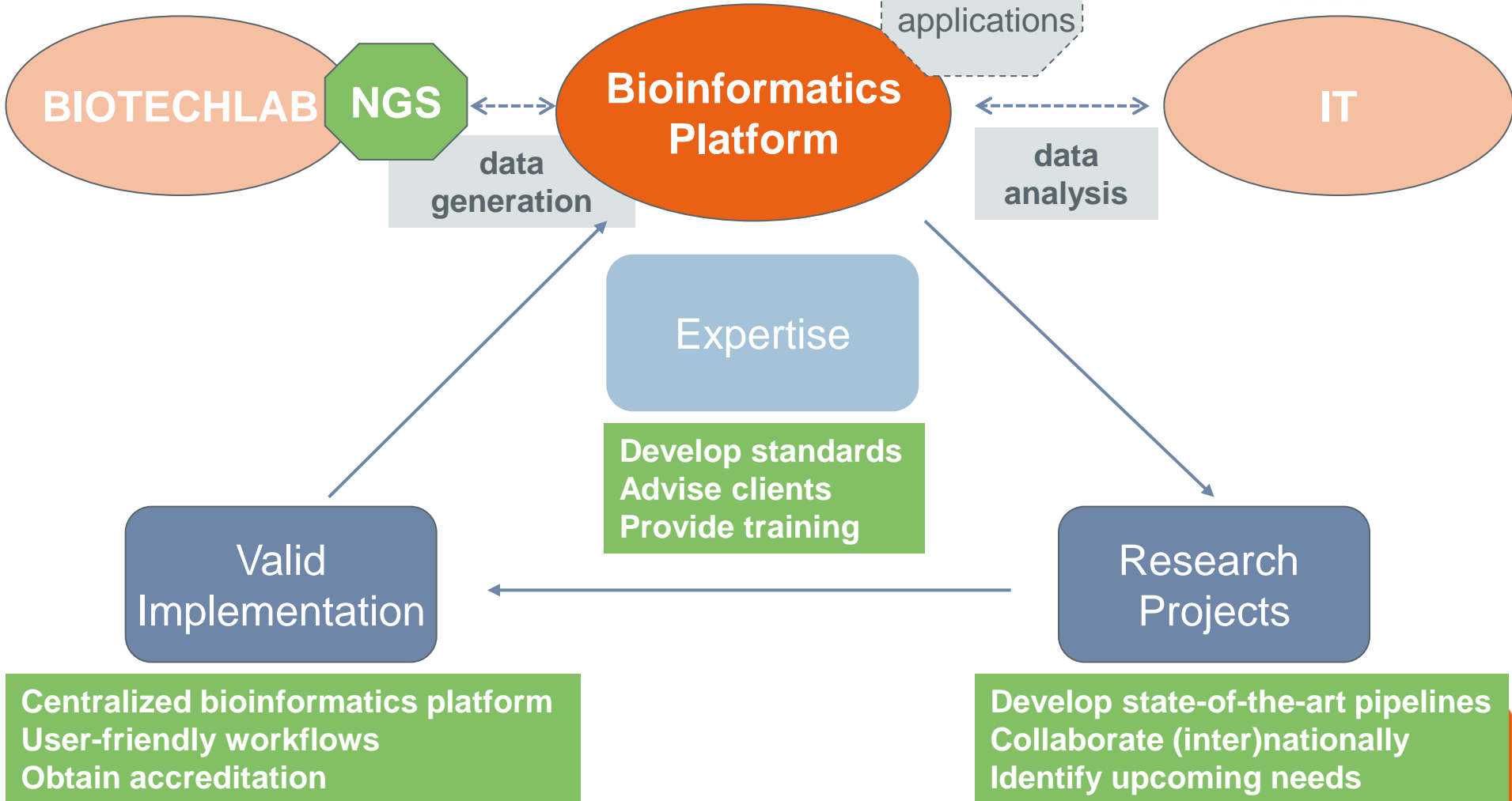
PBB is a transversal scientific service using **molecular biology & bioinformatics** to conduct routine analysis as well as scientific and technologic research.

By building internal and external partnership, it generates new knowledge & customized tools to anticipate present and futures challenges affecting public health

Platform Biotechnology and Molecular Biology



Activities of the bioinformatics platform



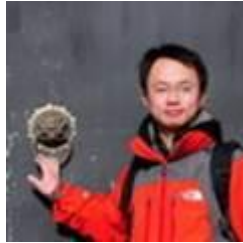
Develop and maintain centralized bioinformatics platform



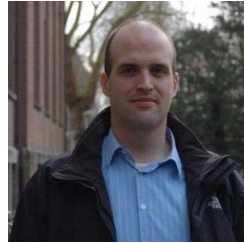
Bioinformatics platform



Bioinformatician
(lead)



Bioinformatician



Bioinformatician



Bioinformatician



Bioinformatician



Software engineer

Development and implementation of user-friendly bioinformatics tools, pipelines and databases



Commercial solutions



In-house developed solutions

Microbial isolates
Mixed samples
Human samples

Bring data analysis into routine



Tools

Staple bioinformatics tools used and adopted by the scientific community
Tools can be combined to make pipelines

'Push-on-the-button' pipelines

Pipelines engineered by the BIOT platform
Case studies tackled according to priorities defined by direction committee

E.g. *Neisseria meningitidis* pipeline

Computational requirements

Tools and pipelines integrated directly into high-performance computational infrastructure WIV-ISP

Validation of standardized & optimized pipelines

Pipelines use validated parameters

User-friendly access for non-experts

Galaxy Workflow Management System for access to non-bioinformaticians

Trade-off between quality and speed of analysis

Different modes of analysis (e.g. surveillance versus outbreak)

Traceability

Automatically updated databases, logging of all parameters and runs

Offer a high-quality service platform



Providing a high-quality service....

Version control

Code review

Basic testing

Technical documentation (bioit Wiki)

User documentation (bioit Wiki)

Several in-take meetings with client to define needs

DTAP principle (**D**evelopment -> **T**esting -> **A**cceptance -> **P**roduction)

Building up the quality system...

2017: Benchmarking to consolidate internal quality system

2018: Obtain certification / accreditation

Combine tools to make pipelines

Tweak parameters

Access using browser (simultaneous usage possible)

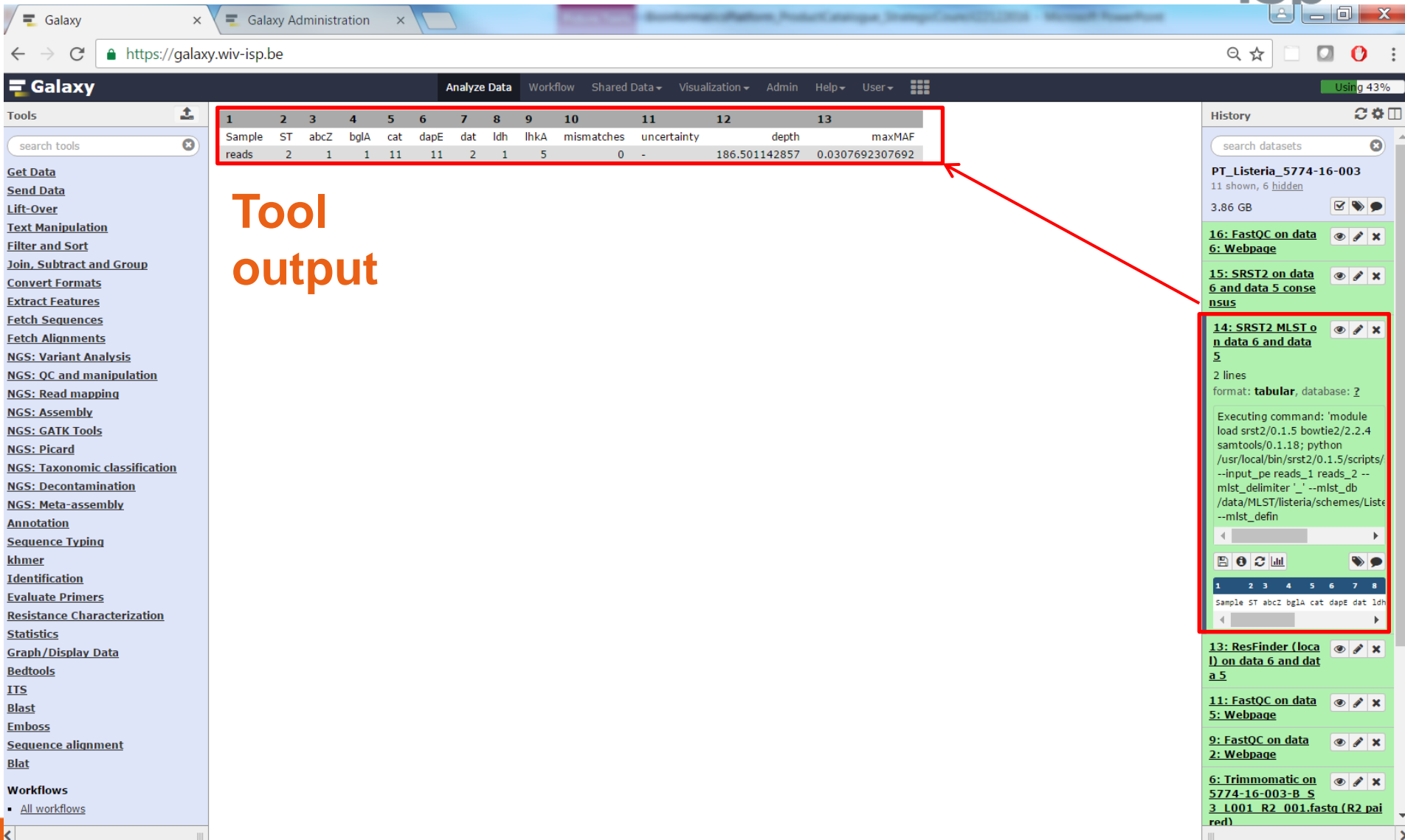
Standard tools

Custom tools

Workflows

The screenshot shows the Galaxy workflow editor interface. The main canvas displays a workflow for plasmid assembly. The workflow starts with two input datasets, each leading to a FastQC tool. The outputs of these FastQC tools are connected to a Trimmomatic tool. The Trimmomatic tool has two input FASTQ files (R1 and R2) and produces several output files. These outputs are then connected to three more FastQC tools. The outputs of these FastQC tools are connected to a SPAdes tool. The SPAdes tool has two input libraries (Forward and Reverse reads) and produces several output files. The interface is annotated with red boxes and text labels. A red box highlights the 'Tools' sidebar on the left, which contains a search bar and a list of tool categories. A red box highlights the 'Details' panel on the right, which shows the configuration for the SPAdes tool. A red box highlights the workflow canvas, which is a grid-based workspace where tools are connected by lines. The text 'Access using browser (simultaneous usage possible)' is overlaid on the top of the workflow canvas. The text 'Standard tools' is overlaid on the left side of the workflow canvas. The text 'Custom tools' is overlaid on the bottom left of the workflow canvas. The text 'Workflows' is overlaid at the bottom center of the workflow canvas.

Combine tools to make pipelines



The screenshot shows the Galaxy web interface. On the left is a sidebar with tool categories. The main area displays a workflow history for 'PT_Listeria_5774-16-003'. A table of tool outputs is highlighted with a red box. A red arrow points from this table to a detailed view of tool '14: SRST2 MLST on data 6 and data 5', which is also highlighted with a red box. The detailed view shows the command used to generate the output.

1	2	3	4	5	6	7	8	9	10	11	12	13
Sample	ST	abcZ	bgIA	cat	dapE	dat	ldh	lhkA	mismatches	uncertainty	depth	maxMAF
reads	2	1	1	11	11	2	1	5	0	-	186.501142857	0.0307692307692

Tool output

```
Executing command: 'module load srst2/0.1.5 bowtie2/2.2.4 samtools/0.1.18; python /usr/local/bin/srst2/0.1.5/scripts/--input_pe reads_1 reads_2 --mlst_delimiter '_' --mlst_db /data/MLST/listeria/schemes/Listeria --mlst_defin'
```


Optimized 'push-on-the-button' pipelines

User-friendly access

Centralized computational infrastructure

Galaxy Analyze Data Workflow Shared Data Visualization Admin Help User

Neisseria Pipeline (Full) Neisseria pipeline (quality control, assembly, resistance characterization and sequence typing) (Galaxy Tool Version 0.1) Options

Sample name

If no sample name is entered, the system will try to detect one based on the input read files. [WARNING] Sample name can NOT be changed afterwards.

Forward reads
33: Sample1-centri_S5_L001_R1_001.Both db seqs

Reverse reads
33: Sample1-centri_S5_L001_R1_001.Both db seqs

Type of analysis
Fast: Typing based on Blastn alignment (DNA) and Blastx alignment (Peptide)
Normal: Typing based on SRST2 read mapping (DNA) and Blastx alignment (Peptide)

ARGannot
Yes No

CARD
Yes No

Regular MLST
Yes No

rplF (50S) Species Identification
Yes No

PorA typing
Yes No

PorB typing
Yes No

FetA typing
Yes No

History
5.2 GB
37: VelvetOptimise r on data 31: Contig Stats
36: VelvetOptimise r on data 31: Contig Stats
35: fqstats on data 31
34: Sample1-centri_S5_L001_R1_001. BWA-MEM Mapping
33: Sample1-centri_S5_L001_R1_001. Both db seqs
32: Sample1-centri_S5_L001_R1_001. Clean seqs
31: Sample1-centri_S5_L001_R1_001. Contaminated seqs
30: Sample1-centri_S5_L001_R1_001. DeconSeq R2 Paired Both db
29: Sample1-centri_S5_L001_R1_001. DeconSeq R2 Paired Clean
28: Sample1-centri_S5_L001_R1_001. DeconSeq R2 Unpaired Contaminant
27: Sample1-centri_S5_L001_R1_001. DeconSeq R2 Paired Both db
26: Sample1-centri_S5_L001_R1_001.

Trade-off between quality and speed (outbreak vs. surveillance)

Automatically updated and traceable databases

Validated & optimized parameters

Optimized 'push-on-the-button' pipelines



Detailed output report

N50: 55509

Assembly (FASTA)

Additional Quality Checks

Median coverage: 75
cgMLST genes found: 1534/1605 (96%)
Reads mapping back to assembly: 98.35%

Test	Forward	Reverse
GC content	Pass	Pass
Mean Q-score drop	Pass	Pass
Average quality score	Pass	Pass
Per base sequence content	Pass	Pass
Maximal N-fraction	Pass	Pass
Sequence length distribution	Pass	Pass

- **Average quality score test:** checks whether the average read quality is above a threshold.
- **GC content test:** checks if the detected GC content is close enough to the expected GC content for this organism.
- **Maximal N-fraction test:** checks whether the maximal N fraction at any read position is below a threshold.
- **Mean Q-score drop test:** checks at which base the mean Qscore drops below a given threshold.
- **Per base sequence content test:** checks whether difference between A-T and C-G is below a threshold at every position. The beginning of the reads can be skipped, as the peaks there can be "normal".
- **Sequence length distribution test:** checks if the fraction of short sequences is below a threshold.

Resistance Characterization

ARGannot

No hits found.

Last updated: 11-12-2016

CARD

Resistance gene	%Identity	HSP/Gene length	Contig	Position in contig	Accession	Alignment
mtxD	99.22	3205 / 3205	NODE_131_length_80077_cov_43.867939	47030..50234	NC_003112	view
mtxE	94.38	1406 / 1405	NODE_131_length_80077_cov_43.867939	45571..46975	X95635	view
mtrC	97.66	1240 / 1240	NODE_131_length_80077_cov_43.867939	50245..51484	NC_003112	view
mtrR	97.32	634 / 634	NODE_131_length_80077_cov_43.867939	51886..52519	NC_011035	view
farB	97.58	1527 / 1527	NODE_147_length_50515_cov_46.459904	15486..17012	NC_003112.2	view
farA	98.65	1115 / 1155	NODE_147_length_50515_cov_46.459904	14348..15462	NC_003112.2	view
macB	97.42	1935 / 1935	NODE_257_length_37940_cov_41.687454	5017..6951	AY768532	view

https://galaxy-dev.wiv-isp.be/root

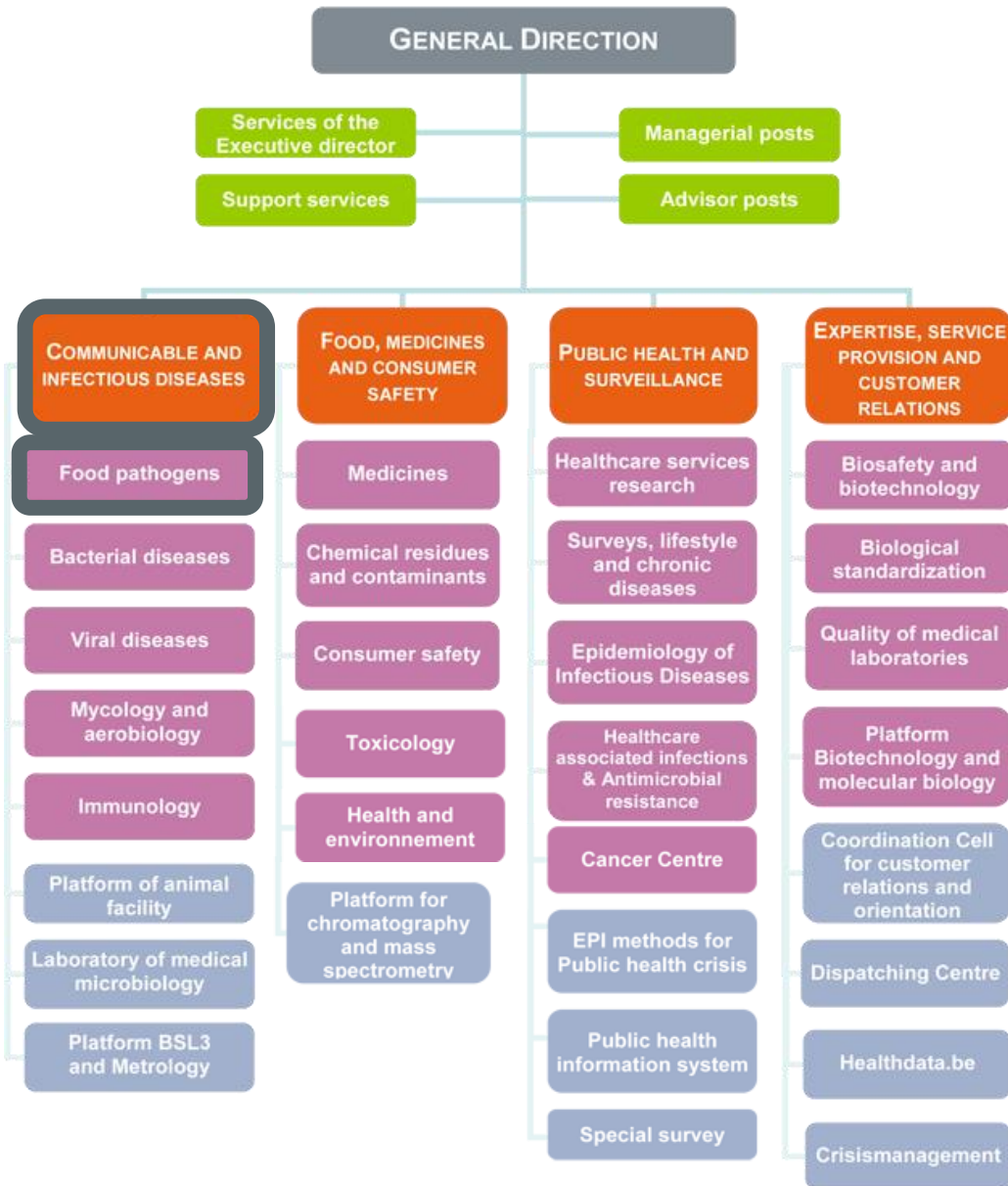
Galaxy

Analyze Data Workflow Shared Data Visualization Admin Help User

Using 74%

- Tools
- Extract Features
- Fetch Sequences
- Fetch Alignments
- Statistics
- Graph/Display Data
- Bert Test Tools
- Annotation
- fgtools
- Genotyping
- khmer
- Decontamination
- Identification
- Logging
- Evaluate Primers
- MLST
- Assembly
- Mothur
- Pipelines
 - Neisseria Pipeline (Full)
Neisseria pipeline (quality control, assembly, resistance characterization and sequence typing)
 - Upload to database
Upload pipeline output to database
 - Sequence typing tree generator
Generates trees based on sequence typing output.
 - Influenza consensus pipeline
Influenza virus pipeline for consensus sequence(s) extraction from NGS data (quality control, assembly/alignment, genome segments sequences extraction)
 - Mumps consensus pipeline
Mumps virus pipeline for consensus sequence(s) extraction from NGS data (quality control, assembly/alignment, genome sequence extraction)
 - Mumps consensus pipeline (stable)
Mumps virus pipeline for consensus sequence(s) extraction from NGS data (quality control, assembly/alignment, genome sequence)

- History
- 11.3 GB
- 136: [TEST] Summary - Neisseria Pipeline (fast) on S15BD01319_S34_L001_R1_001.fastq, S15BD01319_S34_L001_R2_001.fastq
- 135: [TEST] Report - Neisseria Pipeline (fast) on S15BD01319_S34_L001_R1_001.fastq, S15BD01319_S34_L001_R2_001.fastq
- 134: database upload
- 133: database upload
- 132: [TEST] Summary - Neisseria Pipeline (fast) on S15BD01319_S34_L001_R1_001.fastq, S15BD01319_S34_L001_R2_001.fastq
- 131: [TEST] Report - Neisseria Pipeline (fast) on S15BD01319_S34_L001_R1_001.fastq, S15BD01319_S34_L001_R2_001.fastq
- 600.0 KB
- format: html, database: 2
- running neisseria pipeline 2016-12-16 13:10:58,532 - app.loggers.pipelinelogger - INFO - Running pipeline Neisseria_read_trimming 2016-12-16 13:10:58,532 - app.loggers.pipelinelogger - INFO - Job id 8810 2016-12-16 13:10:58,554 - app.loggers.steplogger -
- HTML file
- 130: [TEST] Summary - Neisseria Pipeline (fast) on S15BD01379_S20_L001_R1_001.fastq, S15BD01379_S20_L001_R2_001.fastq
- 129: [TEST] Report - Neisseria Pipeline (fast) on S15BD01379_S20_L001_R1_001.fastq, S15BD01379_S20_L001_R2_001.fastq

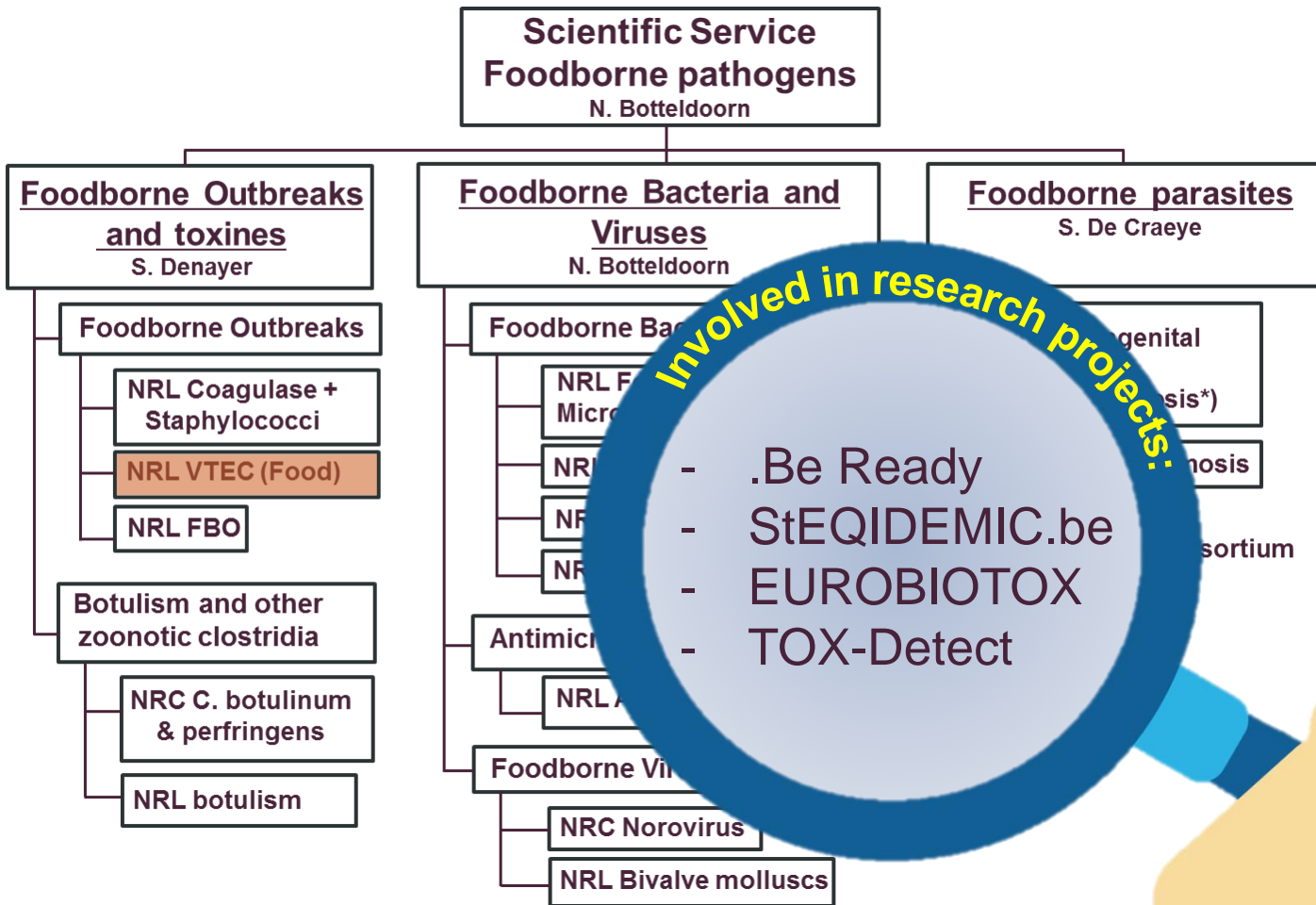


NRL STEC - Belgium

Department communicable and infectious diseases

Food pathogens

NRL STEC - Belgium



NRL STEC - Belgium

E-mail: nrlvti-lnrta@wiv-isp.be

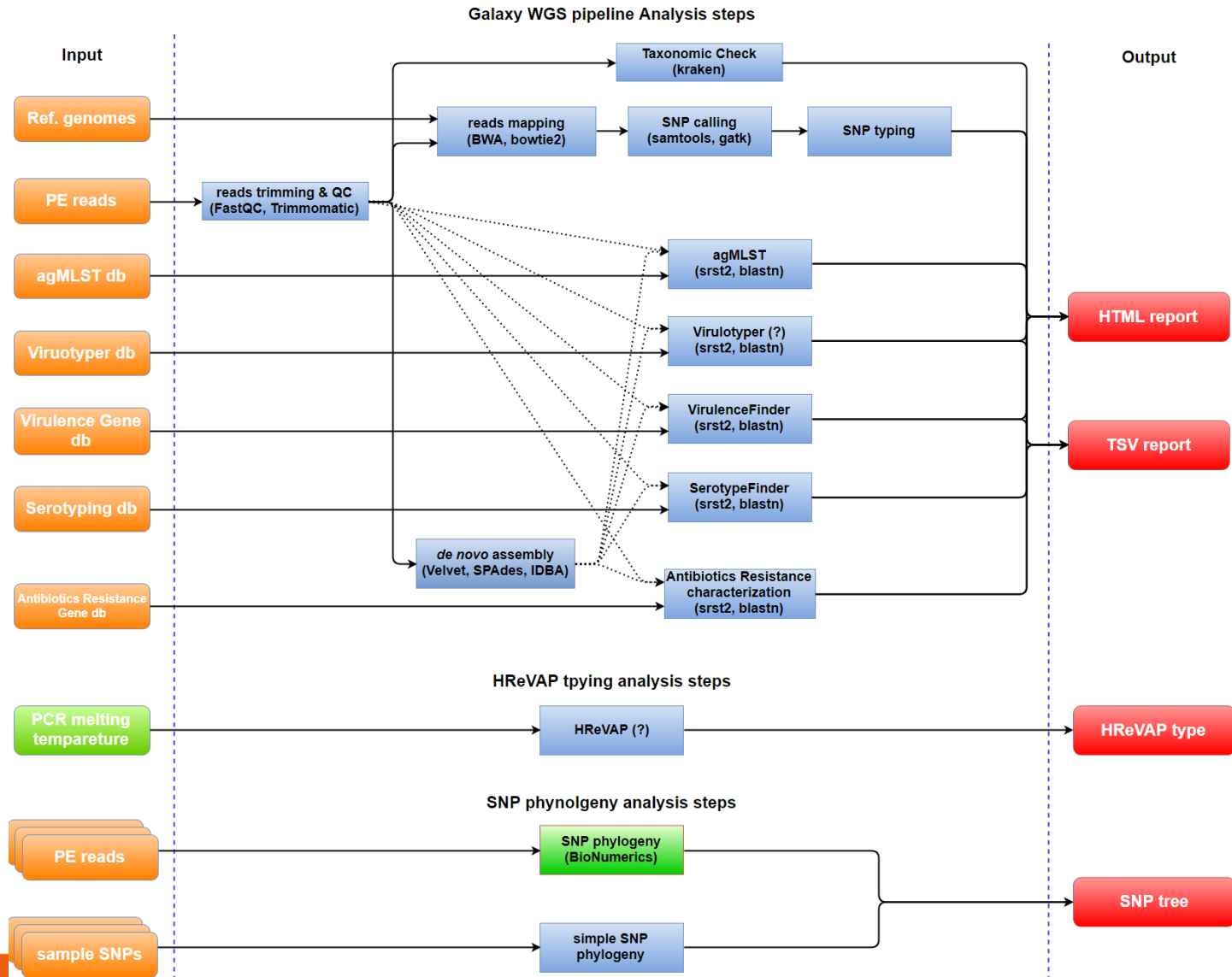


Intake BIOIT needs NRL STEC



- Requirement for a routine pipeline for the analysis of WGS data generated on VTEC and other *E. coli* isolates
- Pipeline should be 'push-on-the-button'
- All functionality should also be available as stand-alone tools
- **Pipeline should be streamlined with the functionality made available by the EURL-VTEC in their ARIES platform**

Analysis BIOIT needs NRL STEC



Data processing



- Quality control:
 - FastQC for generation of raw read reports
 - Trimmomatic for trimming of raw reads
 - FastQC for generation of trimmed read reports
- Contamination check
 - Kraken for identification/confirmation of species based on *kmer* counting, using the entire NCBI RefSeq Microbial database (updated automatically)
- *De novo assembly*
 - SPAdes or Velvet(Optimiser) for assembly of trimmed reads
 - QUAST for quality control of assembly
- ‘Advanced’ quality control
 - A series of custom checks to ensure adequate quality for functional interpretation (%cgMLST genes found, median coverage, N-content...)
 - Provide 3 outcomes: ‘pass’ (all checks passed), ‘warning’ (questionable quality but OK for interpretation), and ‘fail’ (sample should be re-sequenced)

Sequence typing



- MLST, cgMLST, wgMLST, agMLST
 - SRST2 for direct read mapping or BLAST+ for checking assembly
 - MLST (Pasteur/Warwick), cg/wgMLST (Enterobase), agMLST (ARIES) - updated automatically
- Serotyping
 - SRST2 for direct read mapping or BLAST+ for checking assembly
 - SerotypeFinder (DTU/CGE) - updated automatically
- Virulence typing
 - SRST2 for direct read mapping or BLAST+ for checking assembly
 - VirulenceFinder (DTU/CGE) - updated automatically
- Antibiotics resistance
 - SRST2 for direct read mapping or BLAST+ for checking assembly
 - ResFinder (DTU/CGE), CARD (McMaster University), ARG-annot (University of Marseille) - updated automatically
- Plasmid typing
 - SRST2 for direct read mapping or BLAST+ for checking assembly
 - PlasmidFinder(DTU/CGE) - updated automatically

SNP typing/phylogeny



- SNP typing
 - SnapperDB for SNP typing
 - Italian/UK/Belgian database(s) for typing (?)
- SNP phylogeny
 - PHEnix pipeline, CFSAN pipeline, in-house implementation using Samtools for determining phylogeny based on SNPs
 - Output that consists out of a newick tree file and basic visualization of the resulting phylogenetic tree
 - Due to the nature of the these tools, they cannot be integrated in the 'push-on-the-button' pipeline

Others



- HReVAP (ARIES)
- Community requirements?

Input

Sample name

If no sample name is entered, the system will try to detect one based on the input read files. [WARNING] Sample name can NOT be changed afterwards.

Forward reads

Reverse reads

Assembler

Type of analysis

Library kit

Resistance Characterization

ResFinder

ARG-ANNOT

CARD

Virulence Characterization

VirulenceFinder

Serotype Determination

SerotypeFinder

Plasmid Replicon Detection

PlasmidFinder - Enterobacteriaceae

Sequence Typing

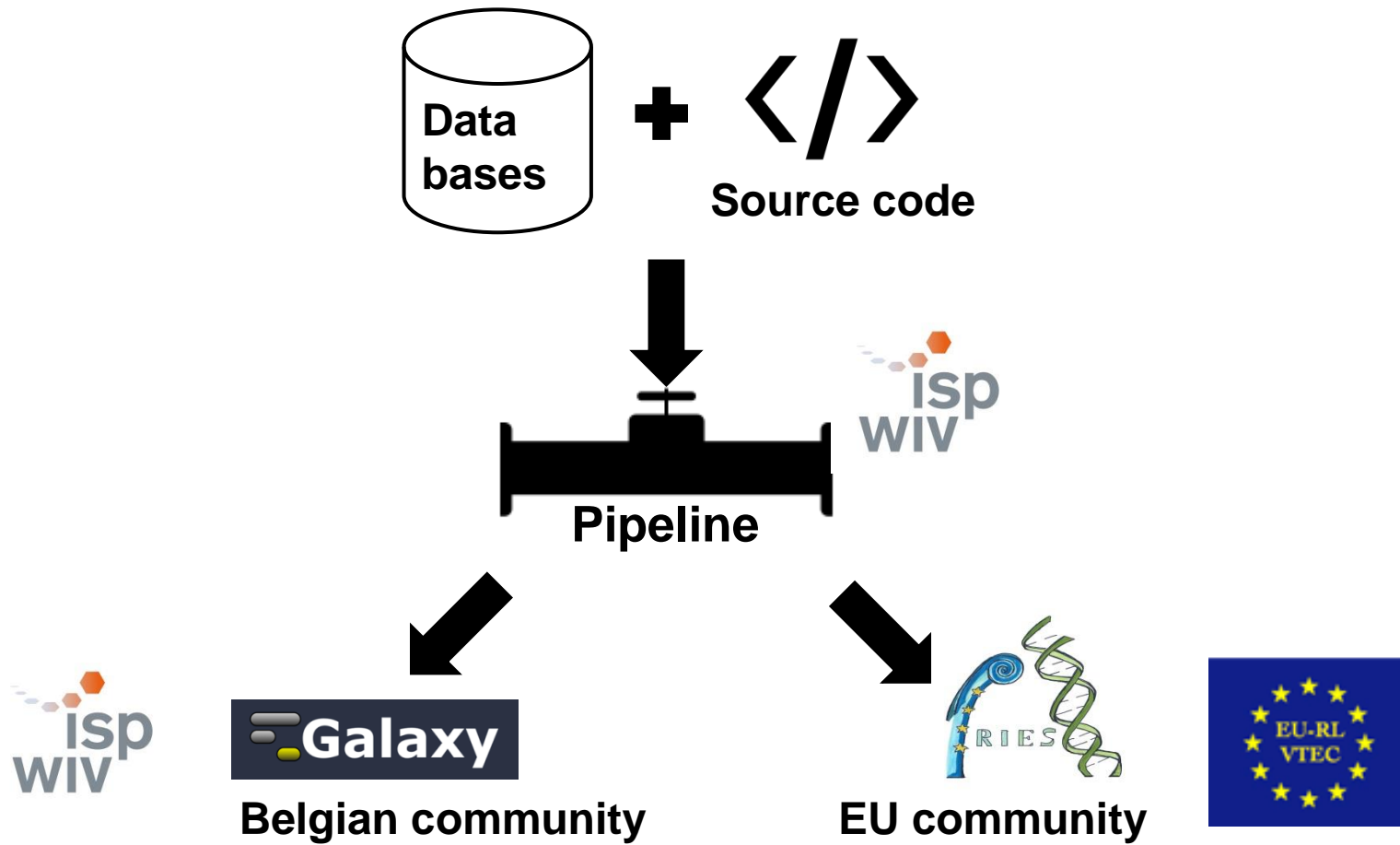
Classic MLST - Pasteur

Classic MLST - Warwick

cgMLST (From Enterobase)



Collaboration ISS and WIV-ISP



**Thank you for your attention!
Questions?**