

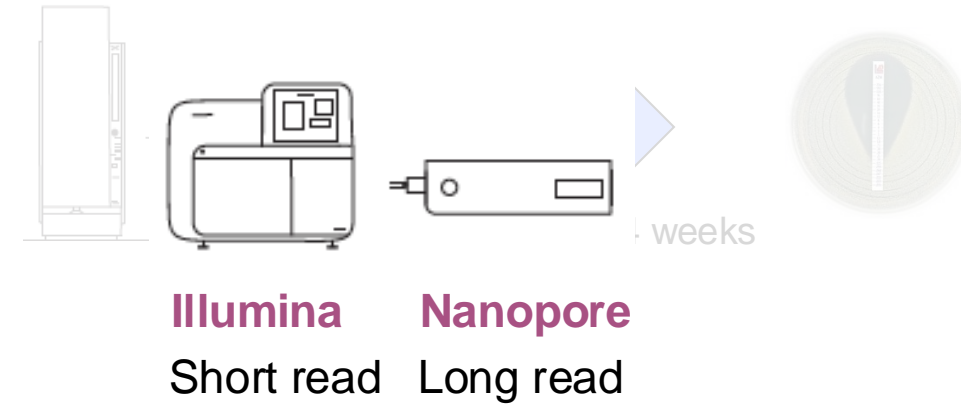
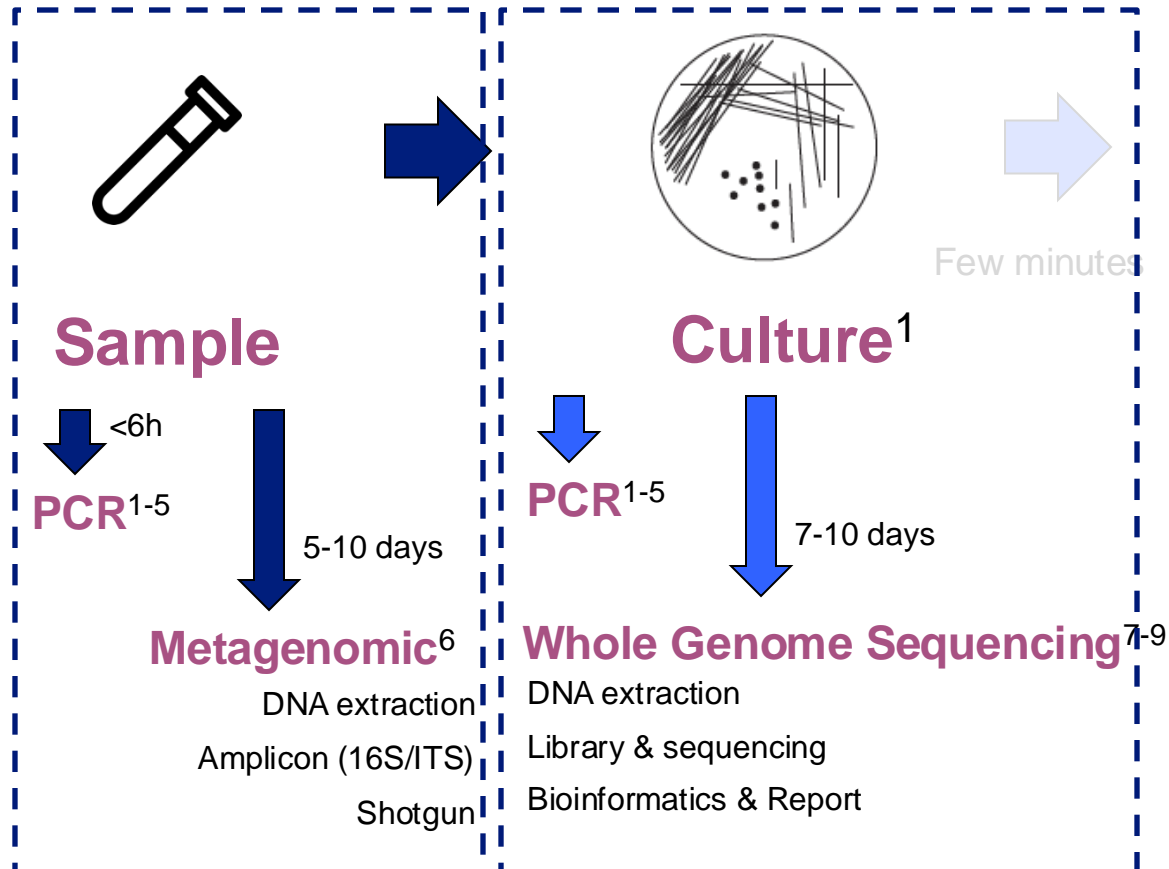


Bio 296: Microbial Bioinformatics

Routine diagnostics and NGS

Tim Roloff

Genotypic axis of diagnostic



→ **Two sequencing techniques**

¹ Hinic V, Amrein I, ... et Egli A J Micro Met 2017; ² Dierig A, Frei R, Egli A, Ped Infect Dis J 2015 ³ Egli A et al. Transpl Infect Dis 2015; ⁴ Osthoff M, ... et Egli, A. Clin Microbiol Infect 2017; ⁵ Weis C, ... Egli A, Borgwardt K, Bioinformatics 2020; ⁶ Egli A, Schmid H, et al. Clin Microbiol Infect 2017; ⁷ Hinic V, Reist J, Egli A J Microbiol Met 2018

Samples from various customers

Customers sending us samples for NGS include

Hospitals (Outbreak investigations, resistance mechanisms)

Reference labs (typing)

Authorities (surveillance, quality management)

Food companies (typing, outbreak investigations)

Pharma companies (strain characterization)

Research groups (various questions)

Standardized workflow in the lab

Standardization is very important for diagnostic workflows

Results can have a big impact for customer/patient

Workflow for typing and detection of resistance gene accredited according to ISO/IEC 17025

- Standard operating procedures (SOPs) for every step
- Internal quality controls (PhiX, QC strains)
- External quality controls (ring trials)
 - Samples sent to different laboratories and results compared
- Audits

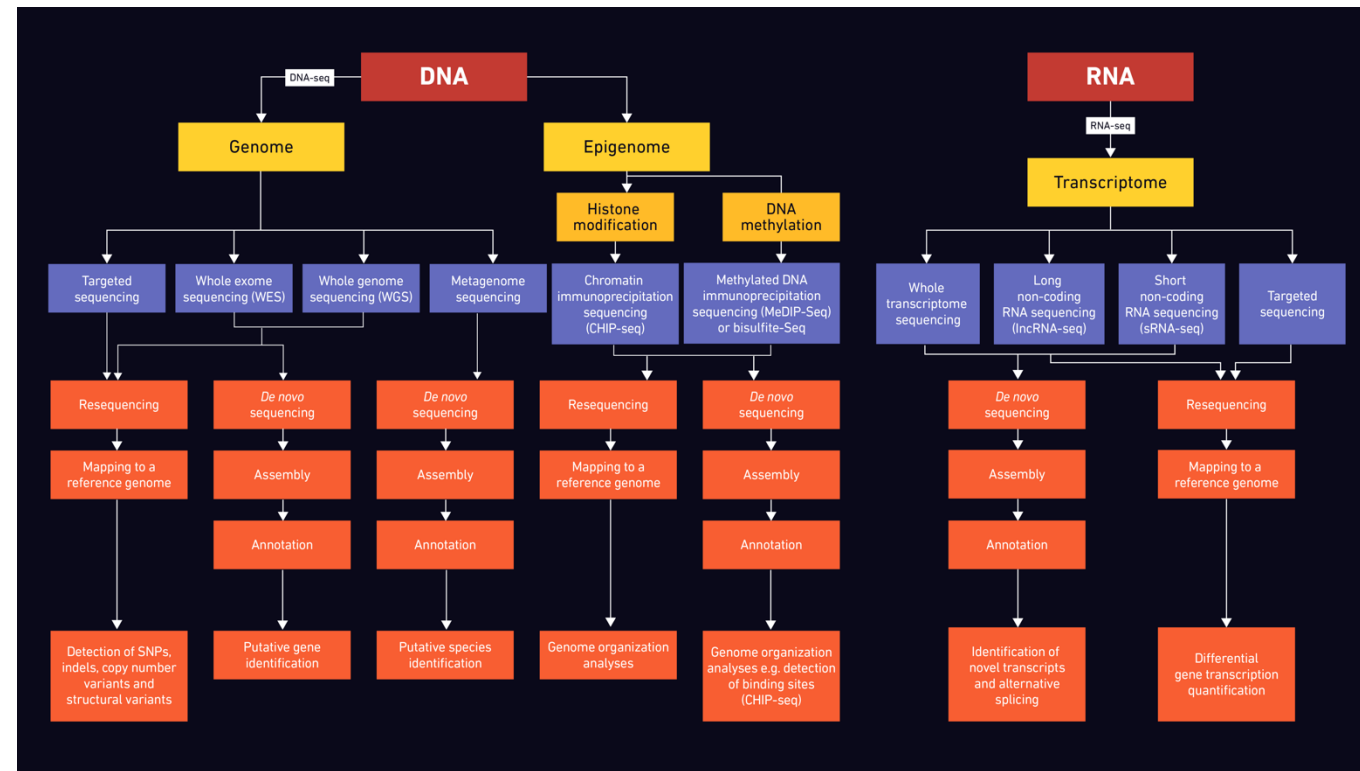


NGS in medical microbiology routine diagnostics

Analyze the genome of a pathogen or bacterial community to study

- Identity (species)
- Resistance genes and virulence factors
- Relationship between isolates
- Replace outdated diagnostics

NGS offers many more possibilities that are not (yet) used in diagnostics



<https://www.technologynetworks.com/genomics/articles/an-overview-of-next-generation-sequencing-346532>

Sample flow for WGS of isolates

Sequencing of cultured isolates:

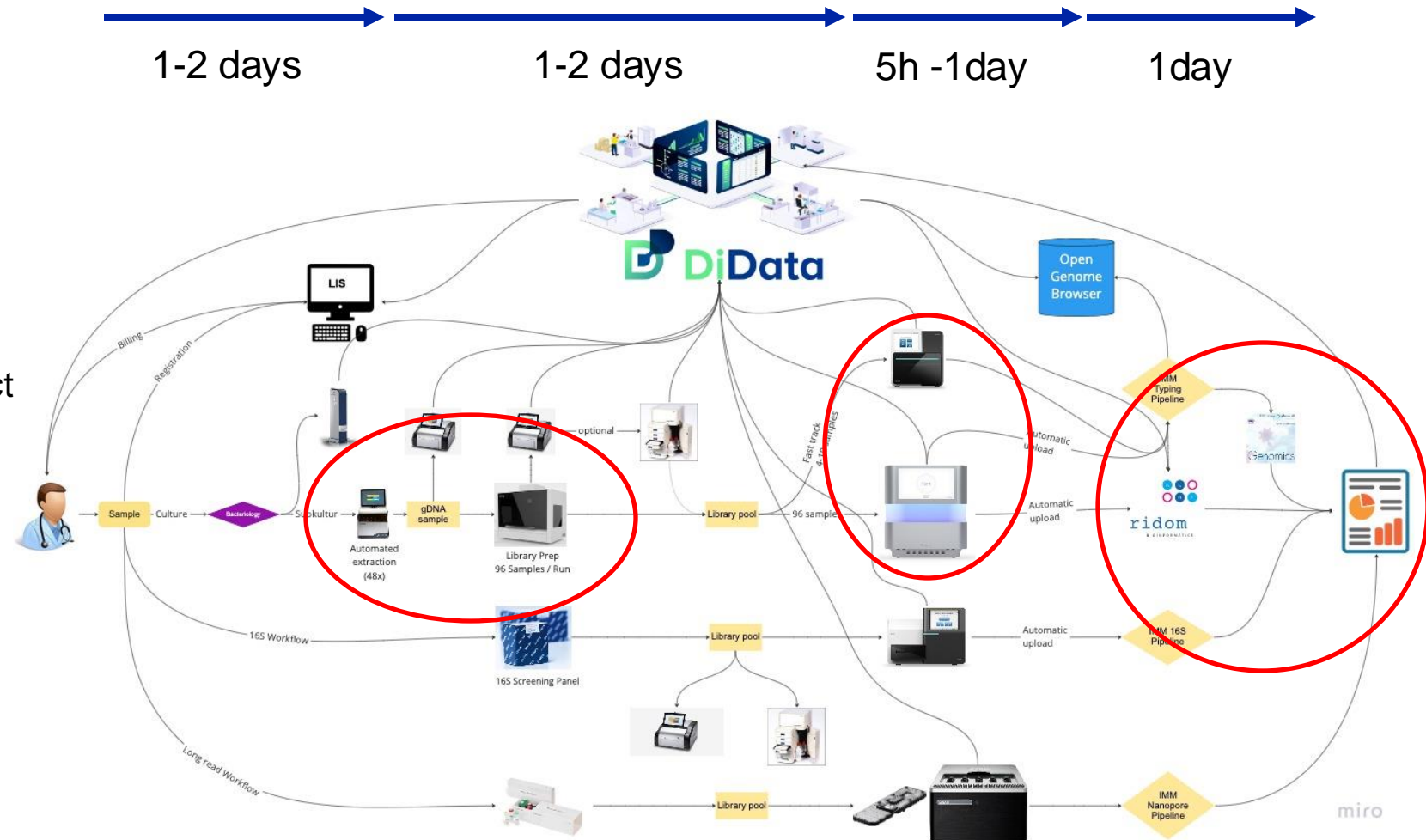
- 96 samples / 5 - 7 days
- 2 days fast track (being set up)

Long diagnostic workflow

Current challenges:

- TAT is too slow to have an impact
- high costs

○ Steps where we can save time and money

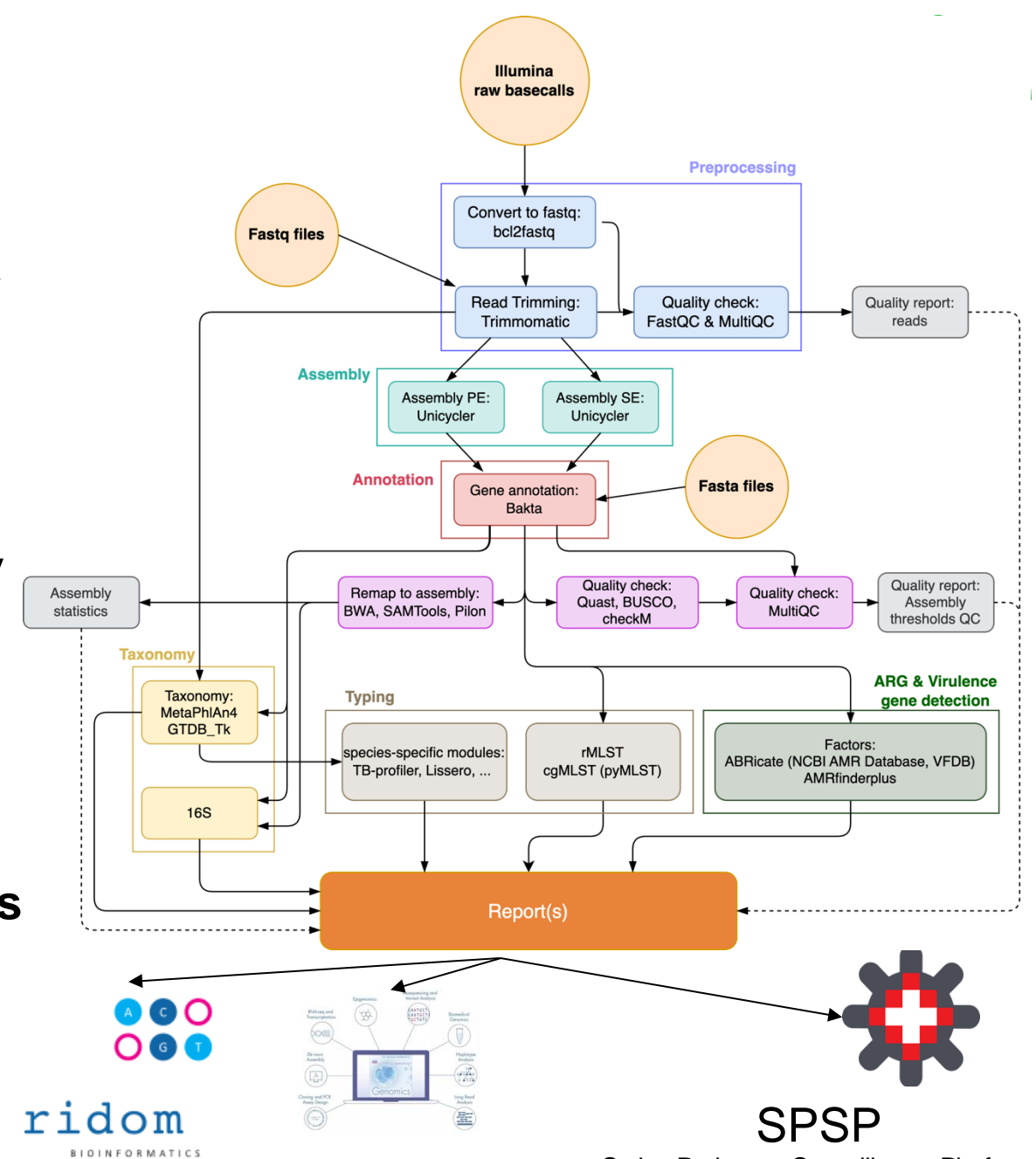


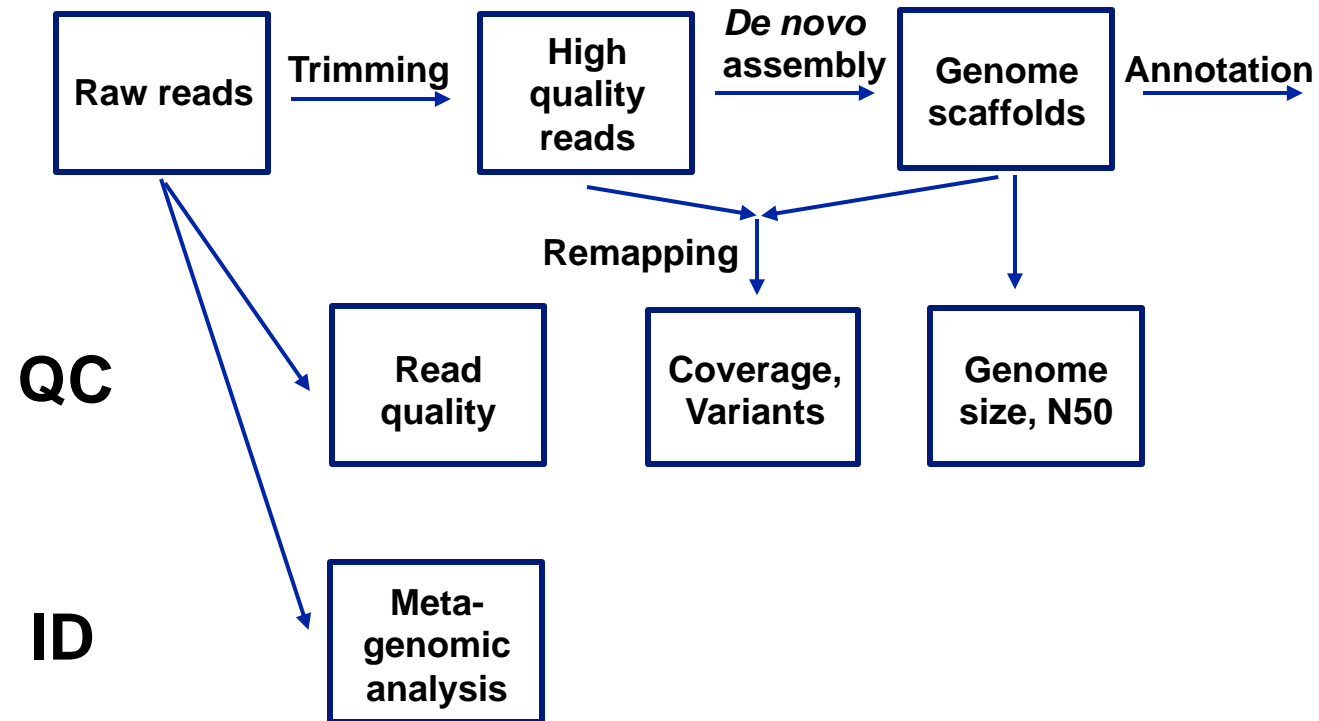
WGS data analysis for isolates: IMMense

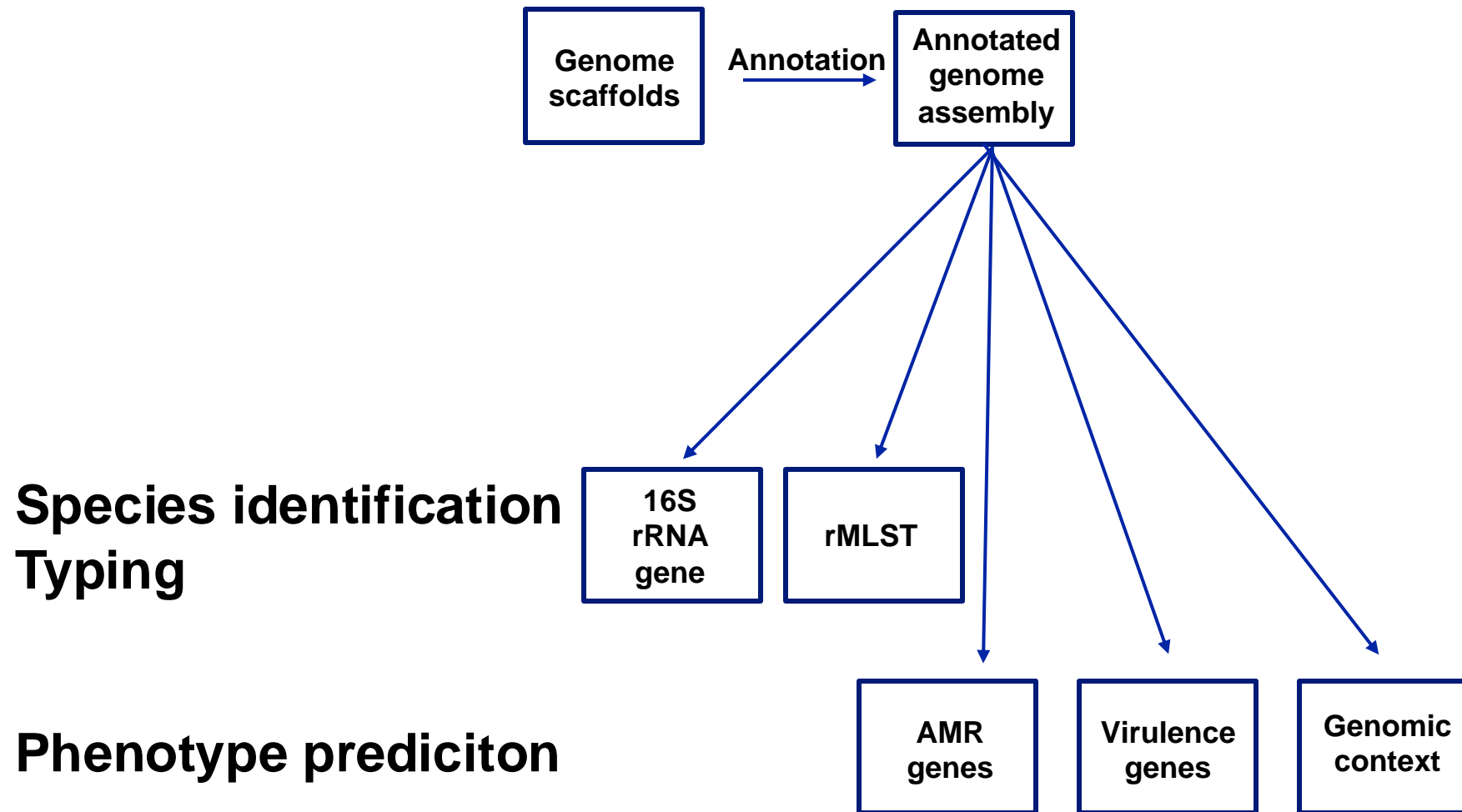
Modular pipeline starting from bcl, fastq or fasta files

- QC, pre-processing, genome assembly, assembly QC, taxonomy, resistance and virulence gene detection
- NextFlow and Singularity for **reproducibility** and **portability**
- Installation on IMM server for patient data security
- Automatically triggered after each sequencing run
- Constantly being extended

In this course we will be using components of this workflow

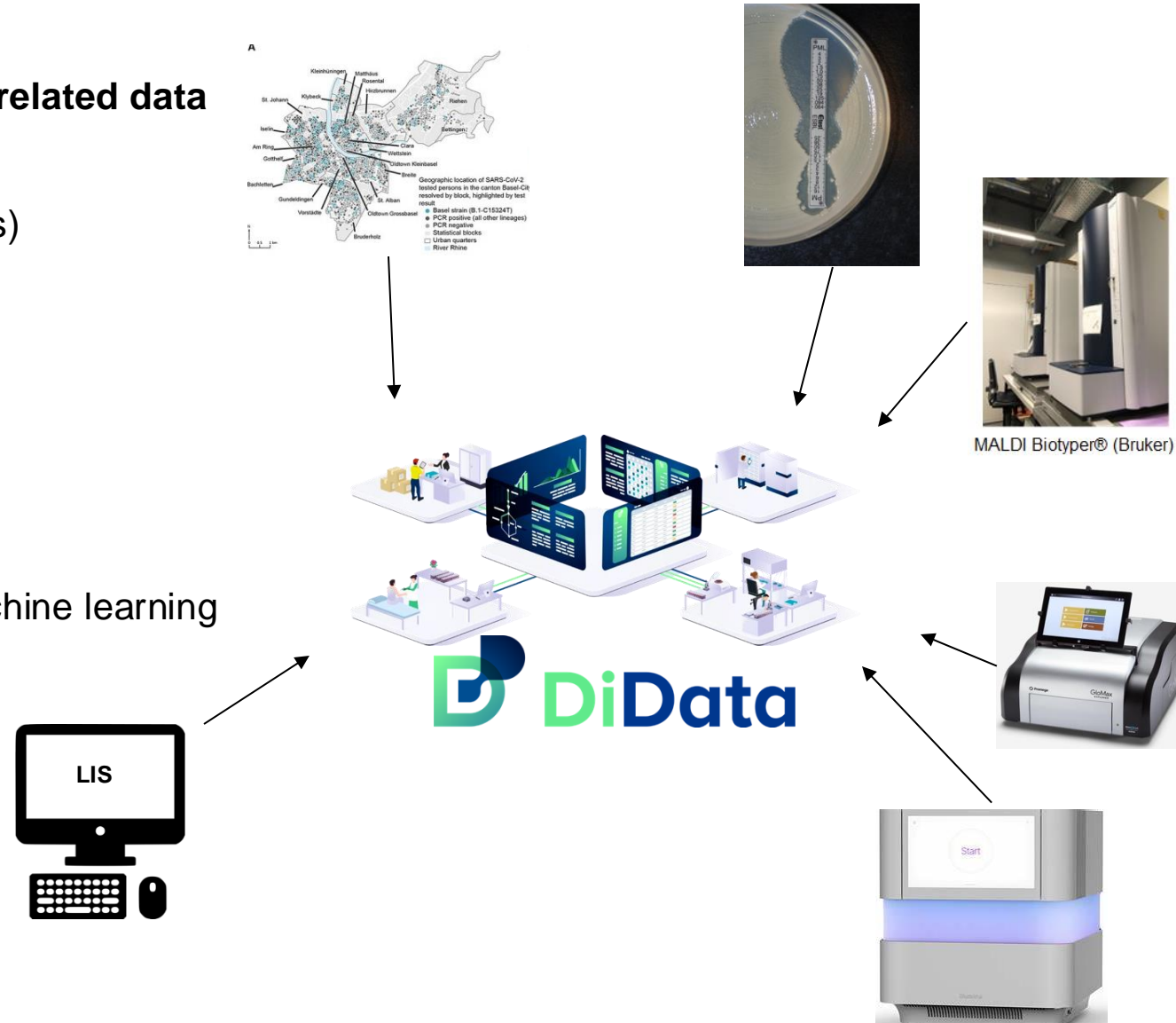






Data integration

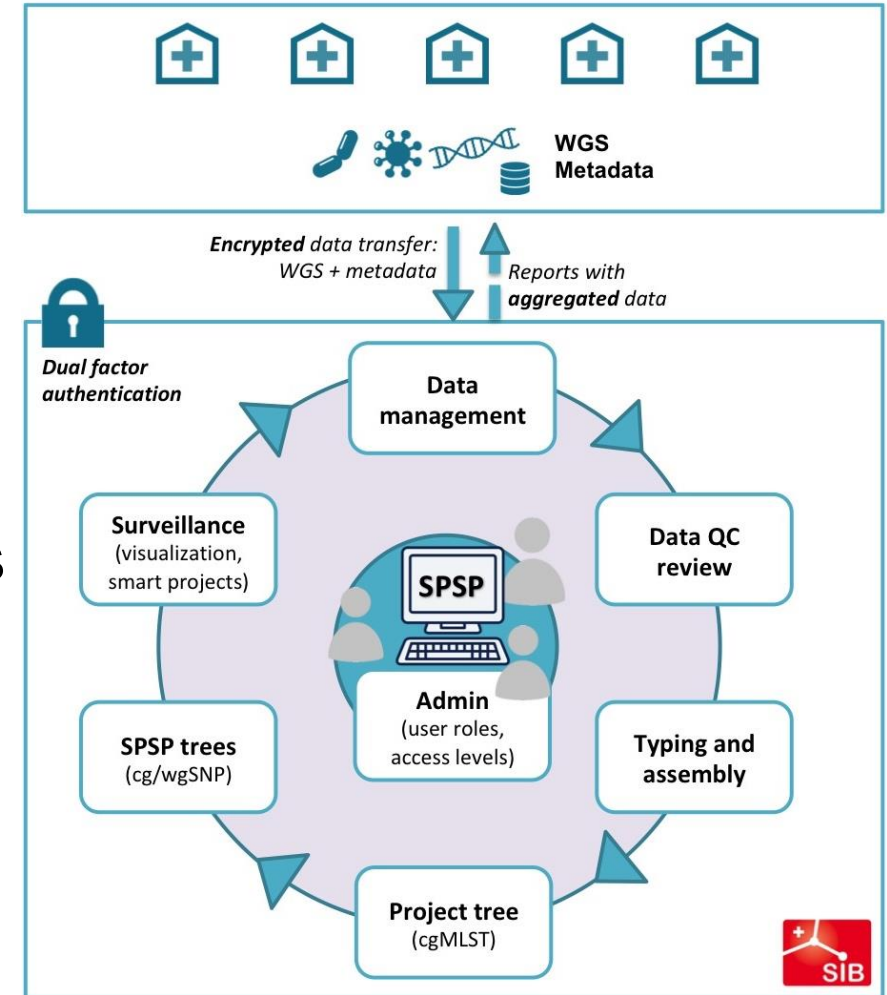
- FAIR data (findable, accessible, interoperable and reusable)
- LIMS system (DiData) as **hub to integrate sample related data**
 - Fastq + QC
 - Assemblies, annotation + QC (21000 assemblies)
 - Patient data from LIS
 - MALDI-TOF MS ID and spectra
 - Anti-microbial resistance profiles e.g. MICs, SIR
 - Epidemiological metadata e.g., geography
- Structured data** allows for data integration and machine learning
 - Phenotypic resistance vs genotype
 - Interpretation of Maldi profiles
 - Biomarkers for clinical phenotypes



Data sharing

Swiss Pathogen Surveillance Platform

- Started with MRSA
- Heavily used for COVID-19 data sharing
- All 5 university hospitals and many other labs connected
- Surveillance beyond cantonal borders



<http://spsp.ch>

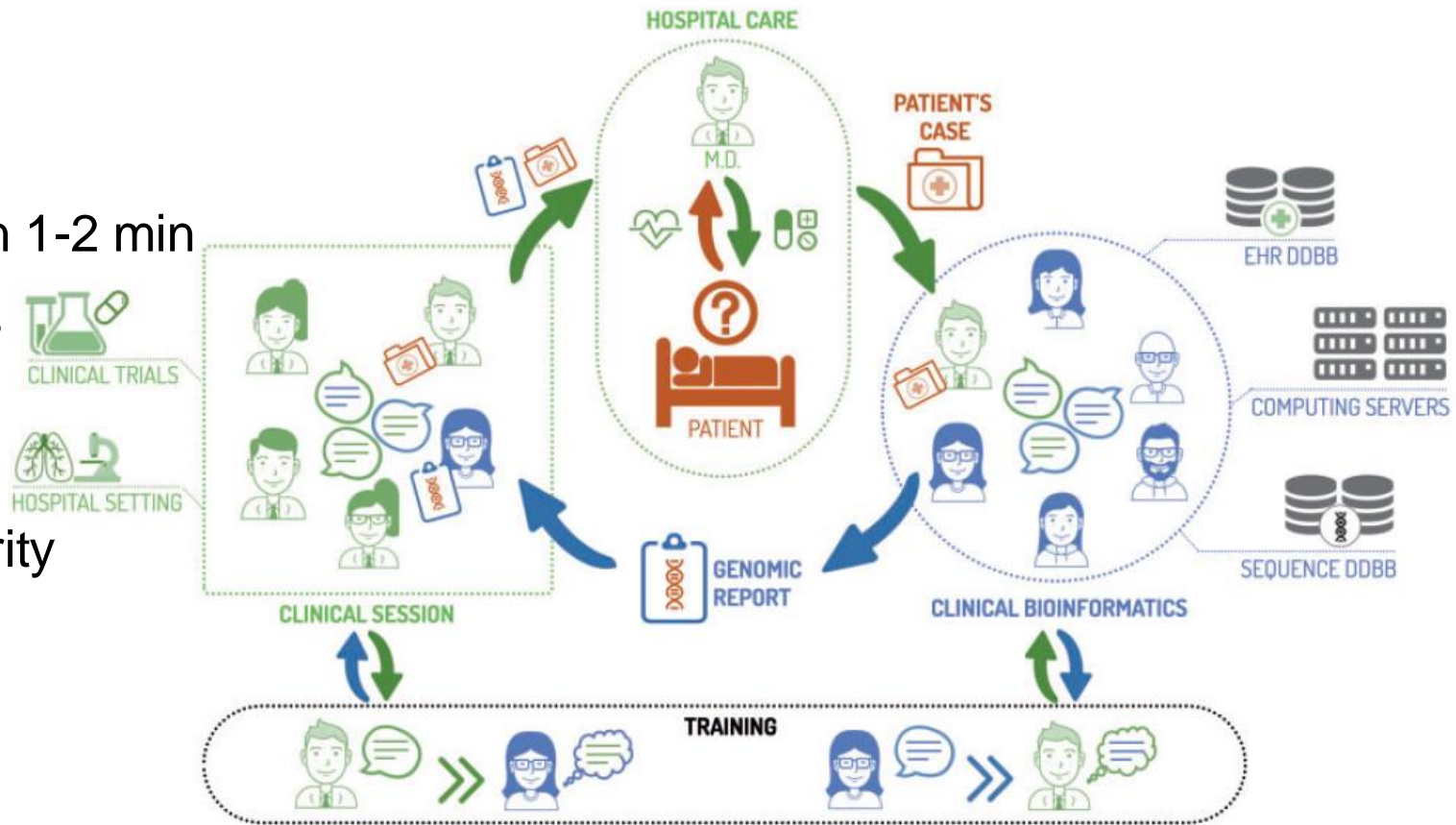
Reporting to interdisciplinary teams

Results reported to various stakeholders

Different levels of complexity

- Clinician needs key message in 1-2 min
- Statistician wants lots of details

Survey across labs and hospitals in Europe to define report granularity for each customer group



Gómez-López *Brief Bioinf* 2017

Carriço *Clin Microbiol Infect* 2018

Standardized report



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Zürich, 29. November 2022

Molekulare Typisierung mittels Genom-Sequenzierung (WGS)

Zusammenfassung

Eine Zusammenfassung der Daten bis September 2022 wird vorgelegt.

Analyst: H. Seth-Smith
Bioinformatik

Freigabe der Resultate
Prof. Dr. A. Egli
Direktor, Institut für Medizinische Mikrobiologie

Summary / Interpretation

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Qualitätssicherung und Durchführung / Methoden und Qualitätssicherung

Gesamtgenom-Sequenzierung (WGS) wurde mit Hilfe eines MiSeq Illumina Sequenziergerätes durchgeführt. Die erhaltenen Sequenzdaten wurden mittels einer bioinformatischen Pipeline (vgl. öffentlicher Qualitätsfiltern kontrolliert (Seth-Smith et al., 2019, Front Public Health, 6694948, 2019 00341)). Die Resultate wurden mit Riken Sequencer (v6.3.4) analysiert durch lineare Schemen (cgMLST.org).

core genome MLST (cgMLST) Methodik vergleicht alle gemeinsamen Gene (Kerngenom) der analysierten Isolate. Dabei wird untersucht, wie viele unterschiedliche Allele die einzelnen Isolate im Genom zueinander aufweisen. Je kleiner diese Anzahl ist, desto näher sind die Isolate zueinander verwandt. Insgesamt werden mehr als 1.400 Gene in den Vergleich eingeschlossen. Esch entsteht eine sehr hohe Auflösung für die Typisierung einzelner Bakterien.

Sequenzierungen und Analysen aller Samples haben die internen Qualitätsstandards erreicht (deckung > 30x Durchschnitt, Genomgröße, Species bestimmt).

Wichtige Auskunft

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Methods

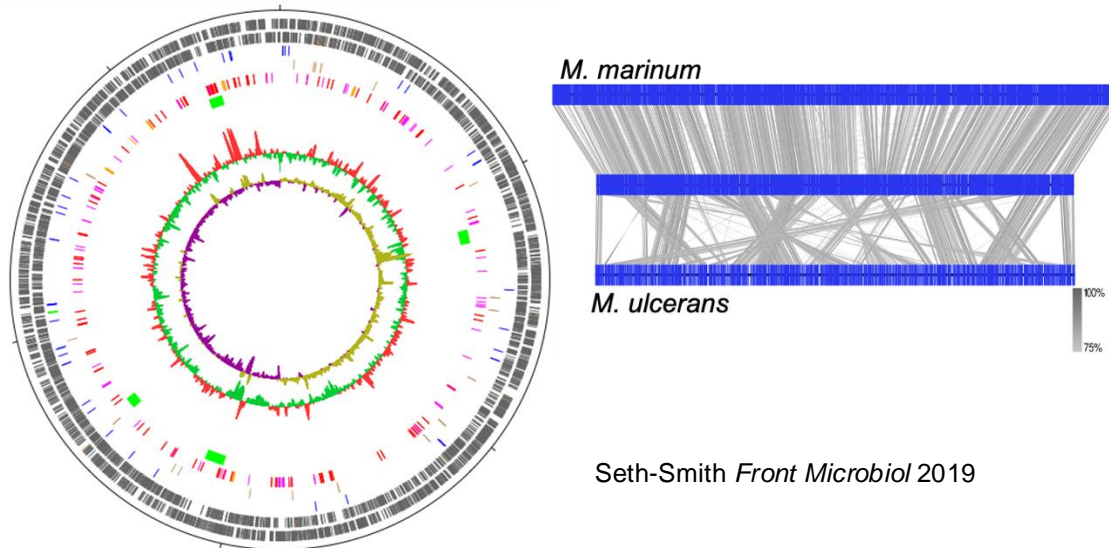


Phylogeny

Sample table

WGS in pathogen identification

- New species *Mycobacterium basiliense* identified and characterised
 - ANI to closest species $\approx 81\%$ ($<95\%$)
 - Digital DDH to closest species $\approx 23\%$ ($<70\%$)



Seth-Smith *Front Microbiol* 2019

- Case study: travel returner with fever
 - *Borrelia persica* identified as pathogen by shotgun metagenomics on blood sample
 - 684 of 7.8M reads mapped to *B. persica* (database assembly AYOT)

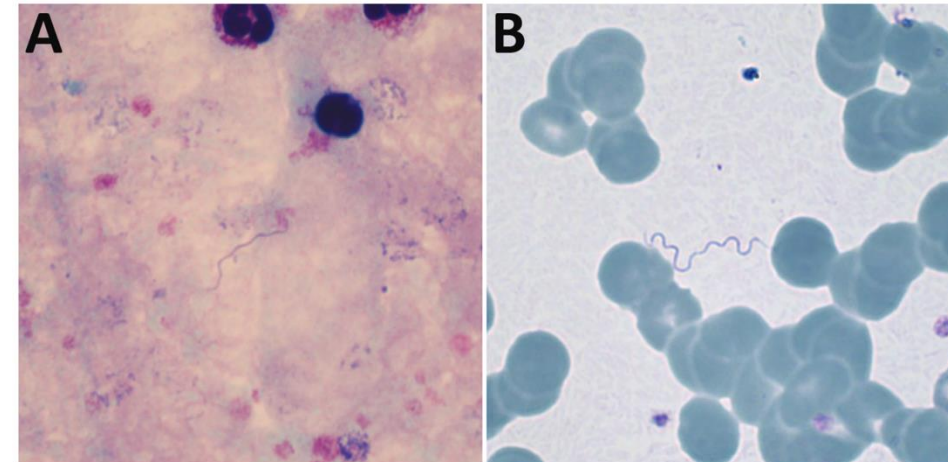


Figure. Giemsa-stained thick (A) and thin (B) blood films, demonstrating extracellular spirochetes. Original magnifications $\times 1,000$.

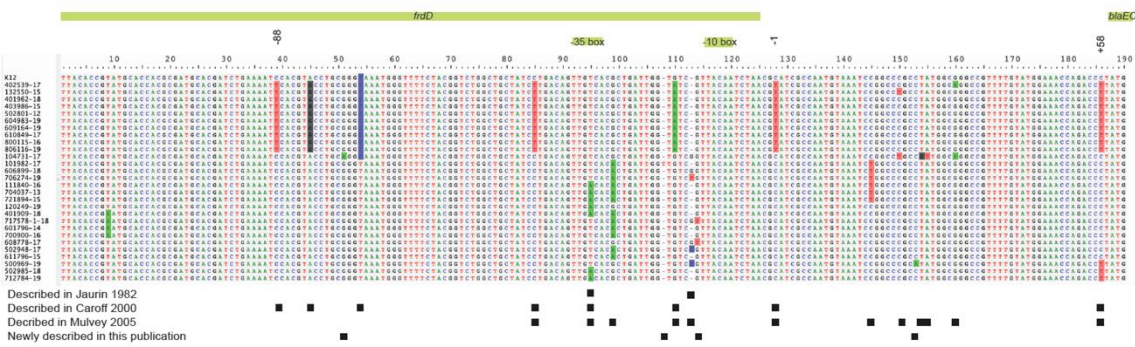
Muigg /Seth-Smith
Emerg Infect Dis 2020

WGS in AMR prediction

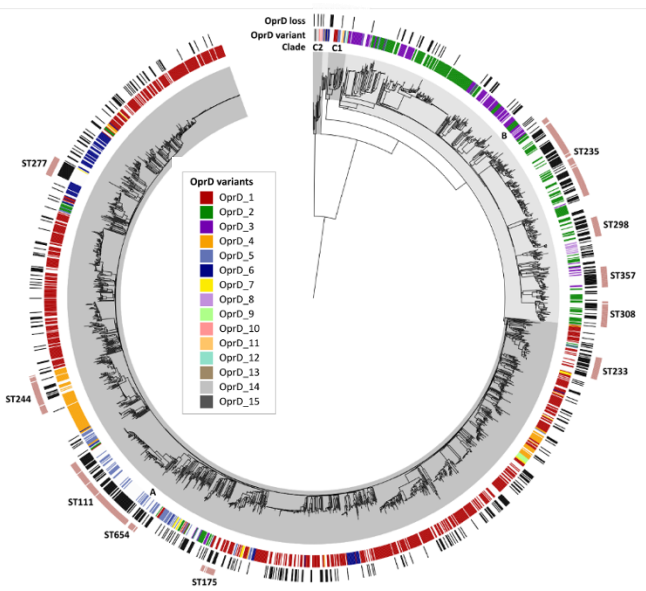


- PorinPredict software developed:
 - Predicts OprD porin loss of function in *Pseudomonas aeruginosa* (meropenemR)
 - Phenotype-genotype comparisons in 1,078 strains, 79 from USB

- Novel *bla*_{EC} promoter mutations found in AmpC phenotype *E. coli* Hinc J Antimicrob Chemother in review

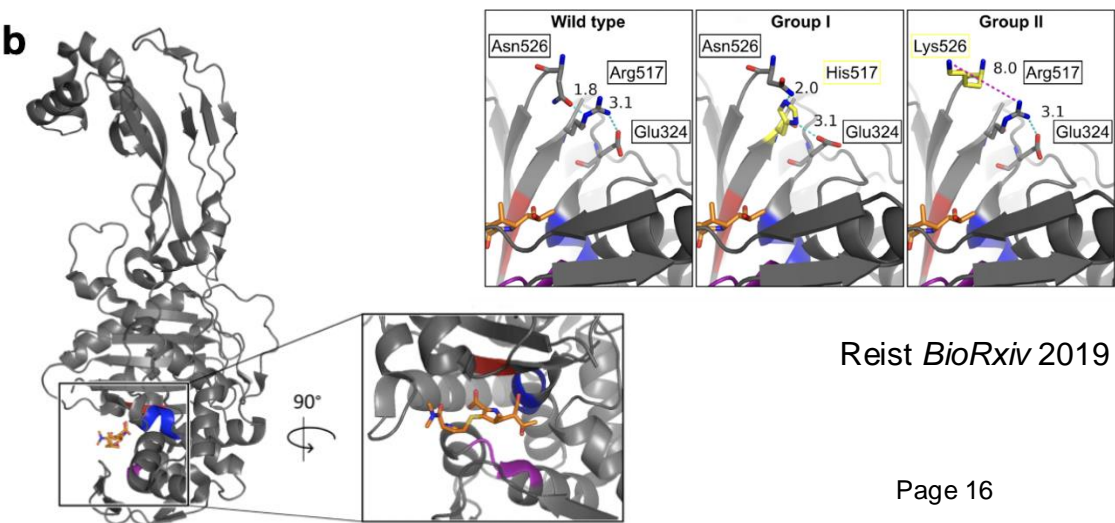


Distribution of porin loss in 2088 genomes



Frameshifts / terminations / promoter mutations

- Phenotypic impact of amino acid substitutions in *Haemophilus influenzae* *pbp3*



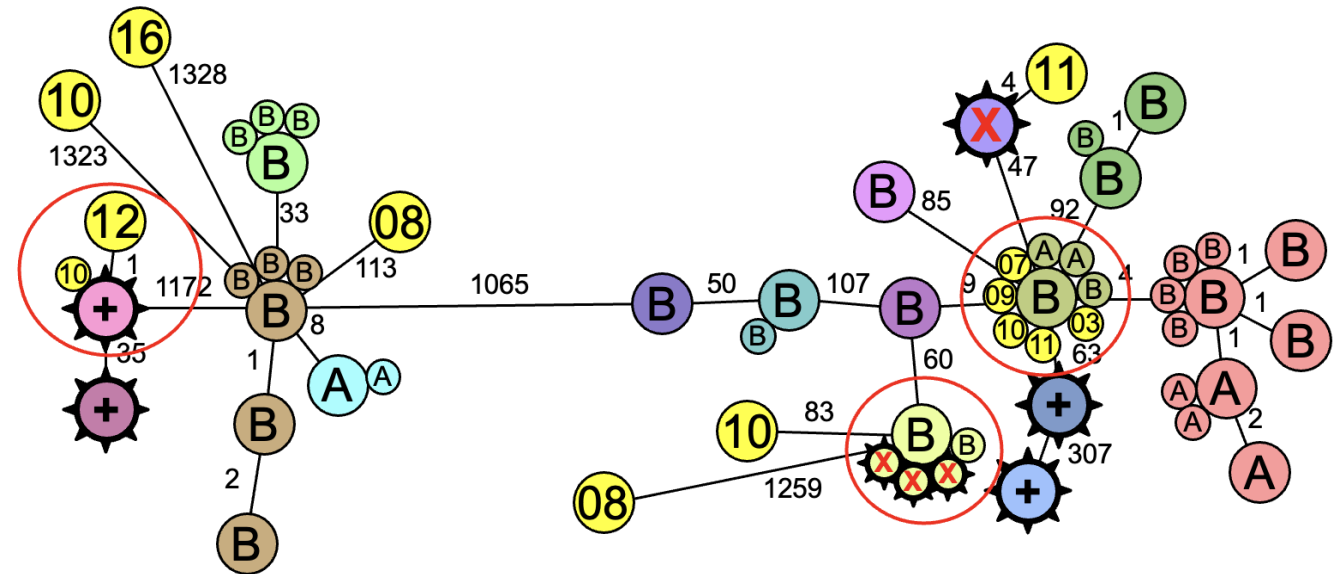
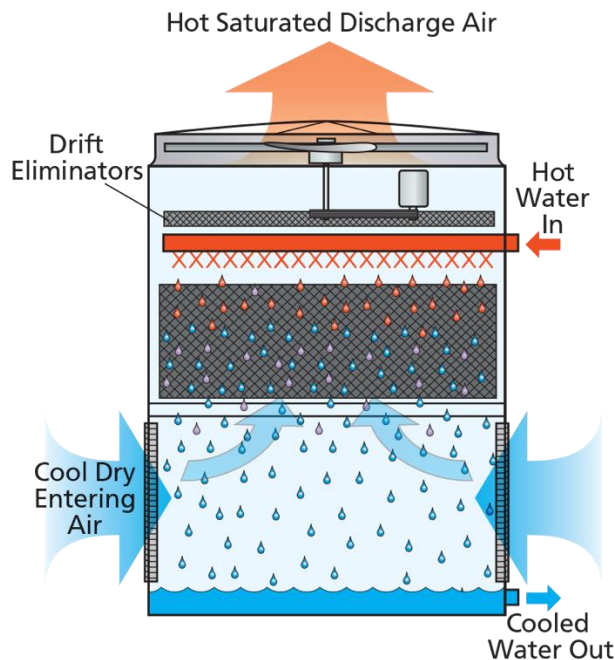
Reist BioRxiv 2019

Biggel Microbiol Spectrum in press

WGS to solve outbreaks: *Legionella pneumophila*

Legionella pneumophila infections from same urban district

- *Legionella pneumophila* detected in cooling towers
- Cooling towers (A and B) have diverse strains, but also share similar strains
- Three 2017 outbreak isolates (X) identical to cooling tower B isolates
- Clinical isolates from previous years are closely related to other cooling tower strains



cgMLST Sequence types (Cluster types)



Isolates 2017 **X** Isolates 4058  Isolates last decade

Wüthrich D et al. *Eurosurveill* 2018

WGS in outbreaks: *Corynebacterium diphtheriae* 2022

Increase in diphtheriae cases in refugee camps across Europe

- Determination of MLST, clusters, toxin presence, ARG from the genome assembly
- Visualised in Ridom Seqsphere+, superimposed on Minimum Spanning Tree



NGS in routine diagnostics - summary

- Used for identification, outbreak investigation, surveillance and resistance / virulence prediction
- Highest resolution for outbreak investigations and surveillance
- Interdisciplinary team needed (BMAs, bioinformaticians, clinicians/specialists)
- ISO accredited workflow
- Proper communication of results is crucial
- Rather expensive and slow diagnostic tool (work in progress)
- Proper data storage needed to make most out of the data, e.g. with machine learning (FAIR principles)

