Pop Rocks

The Explosive Potential of Sequencing

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Slides live at:
www.k-florek.net/talks

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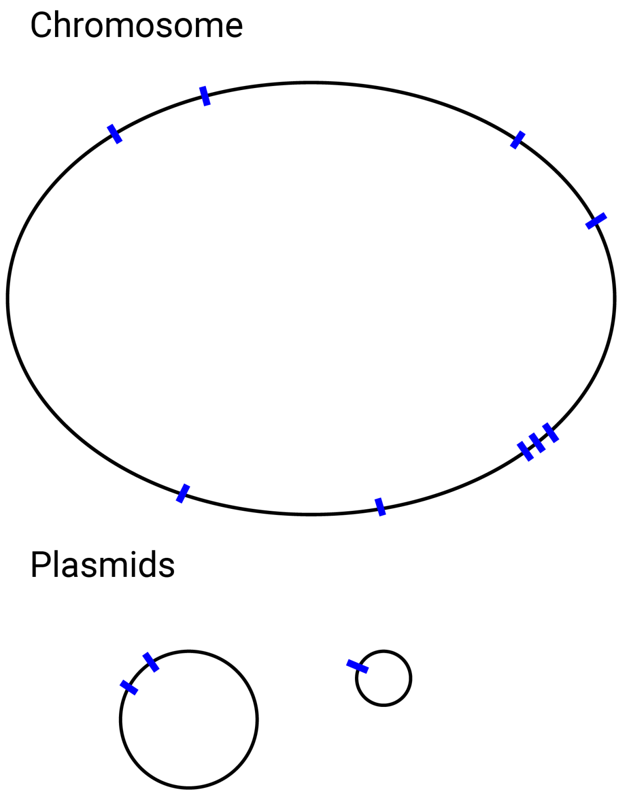
Wisconsin State
Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Advances in Genomic Characterization

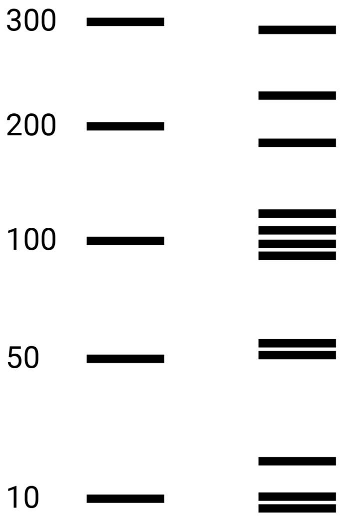
PFGE - Traditional Genotyping

Bacterial Isolate



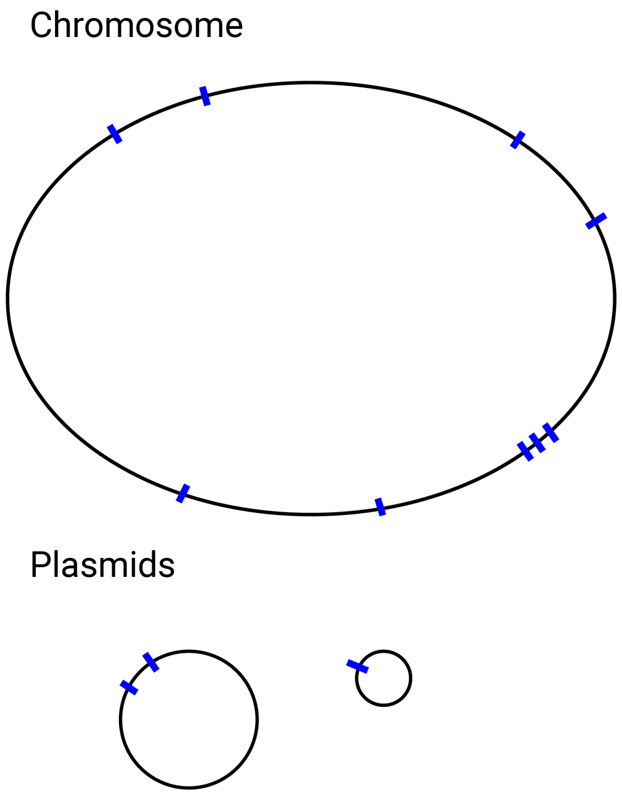
Distribution of Fragments

PFGE

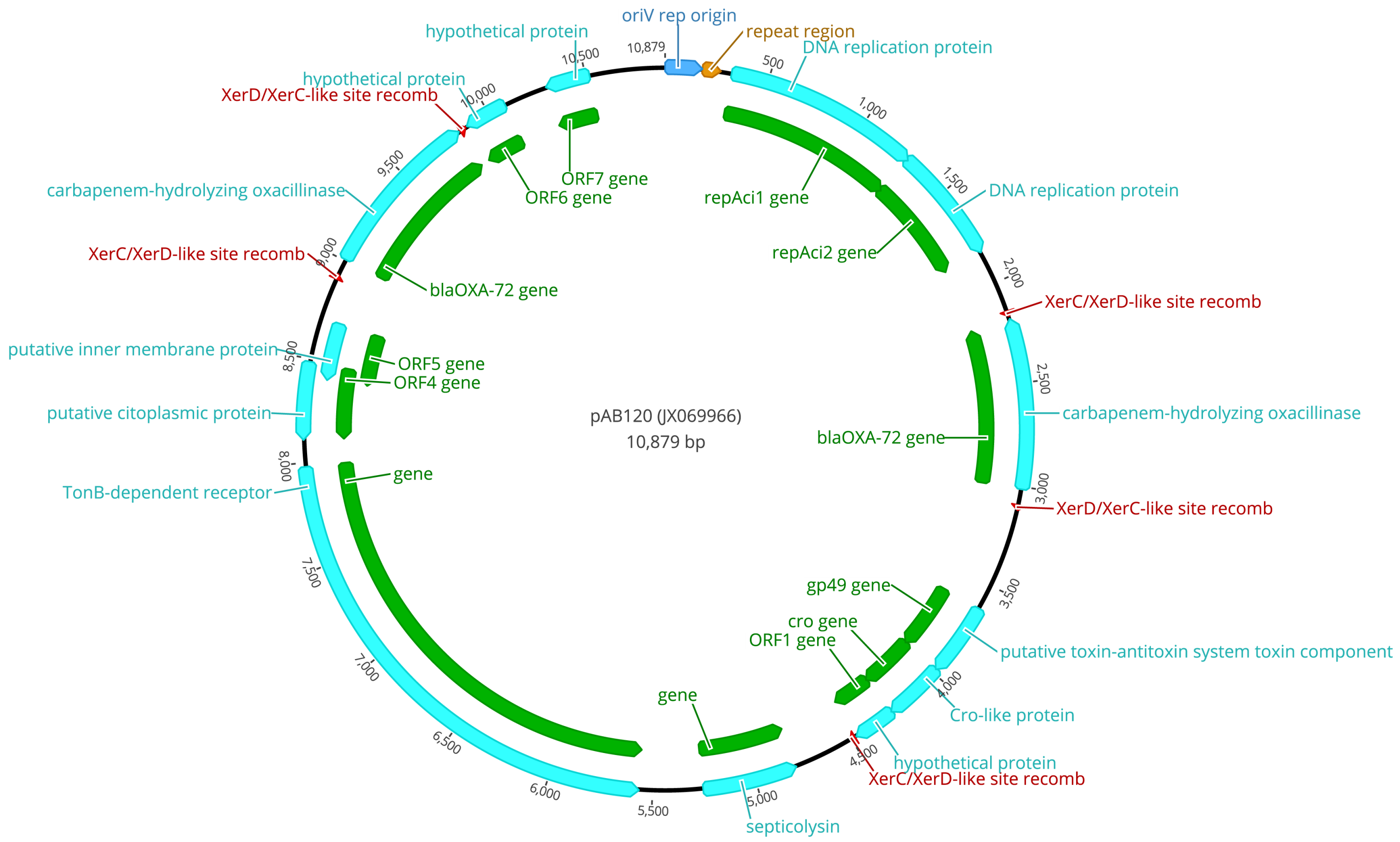


Increasing the resolution of genomic information

PFGE



Whole Genome Sequencing (WGS)



Where we have been and where we are going

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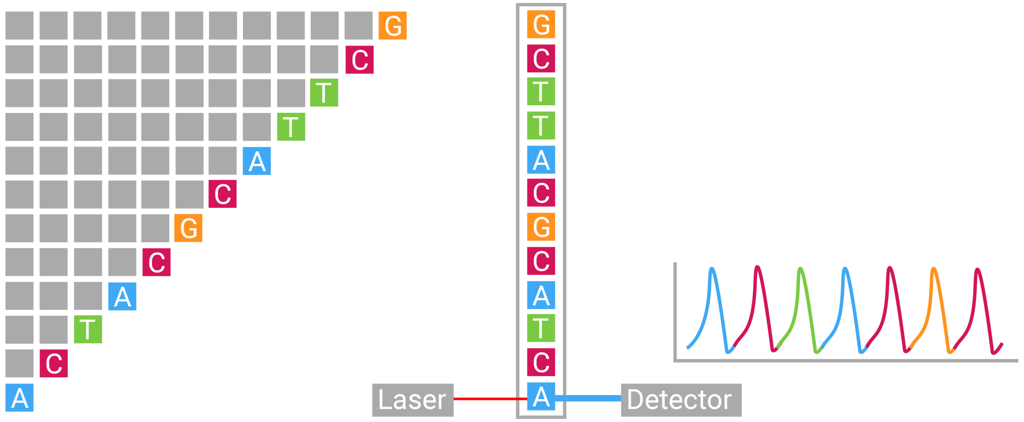
Sanger Sequencing



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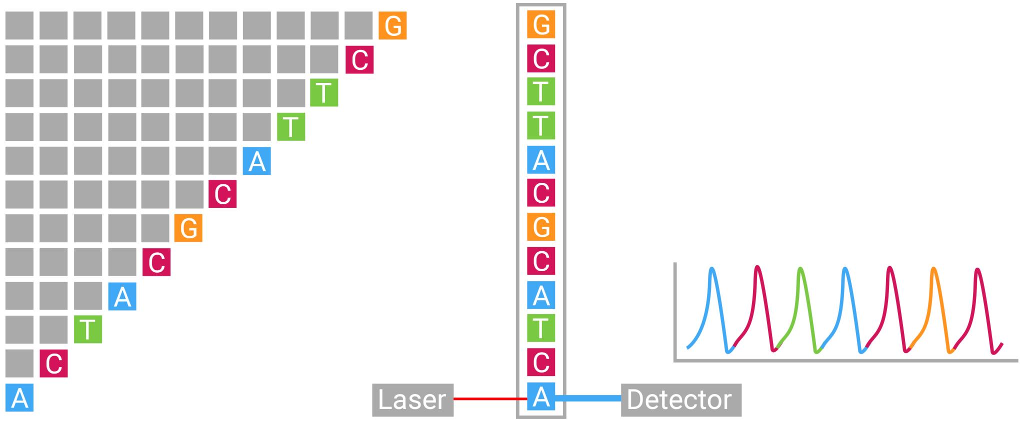
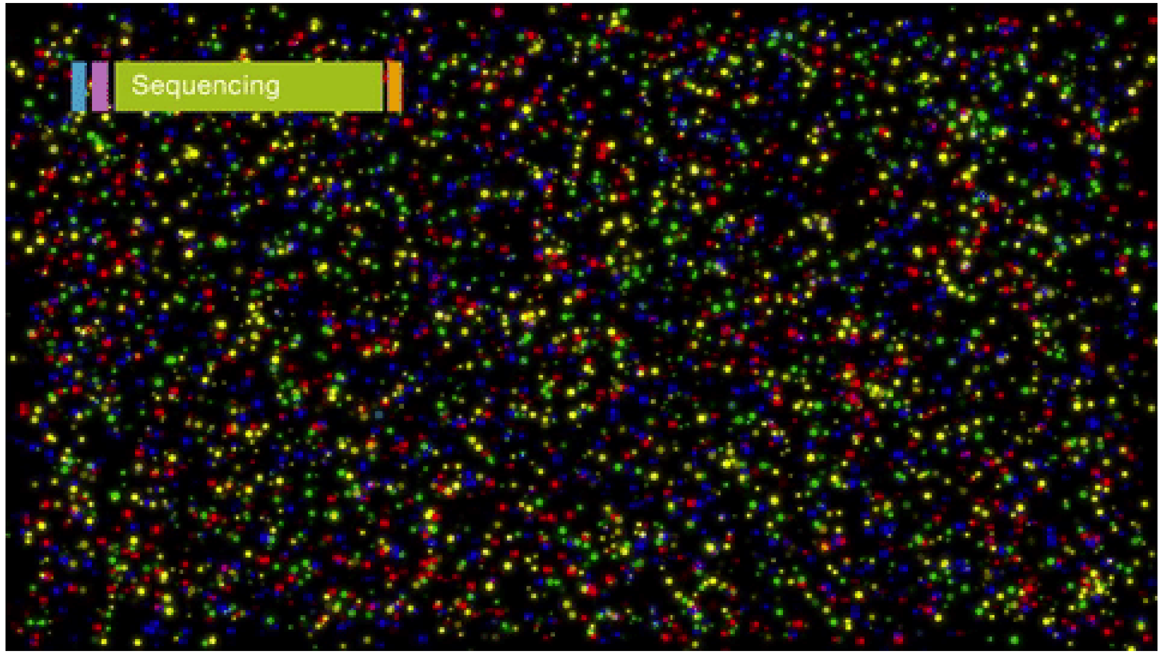
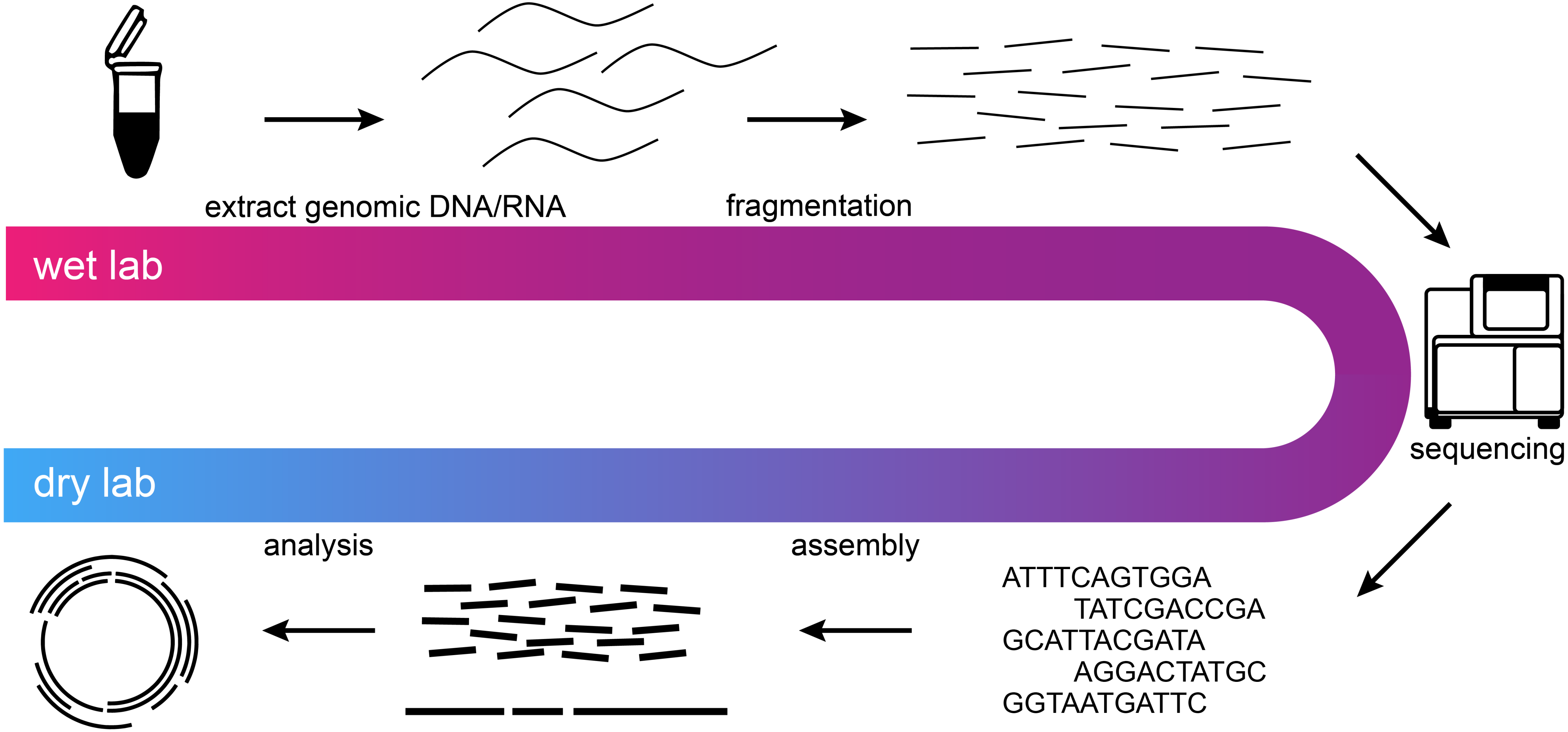


Next Generation Sequencing (Illumina)





extract genomic DNA/RNA

fragmentation

wet lab

sequencing

dry lab

ATTTCAGTGGA
TATCGACCGA
GCATTACGATA
AGGACTATGC
GGTAATGATTC

assembly

analysis

Increases in data requires advanced analyses (MiSeq)

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- 15,000,000,000 ATGC's generated per sequencing run

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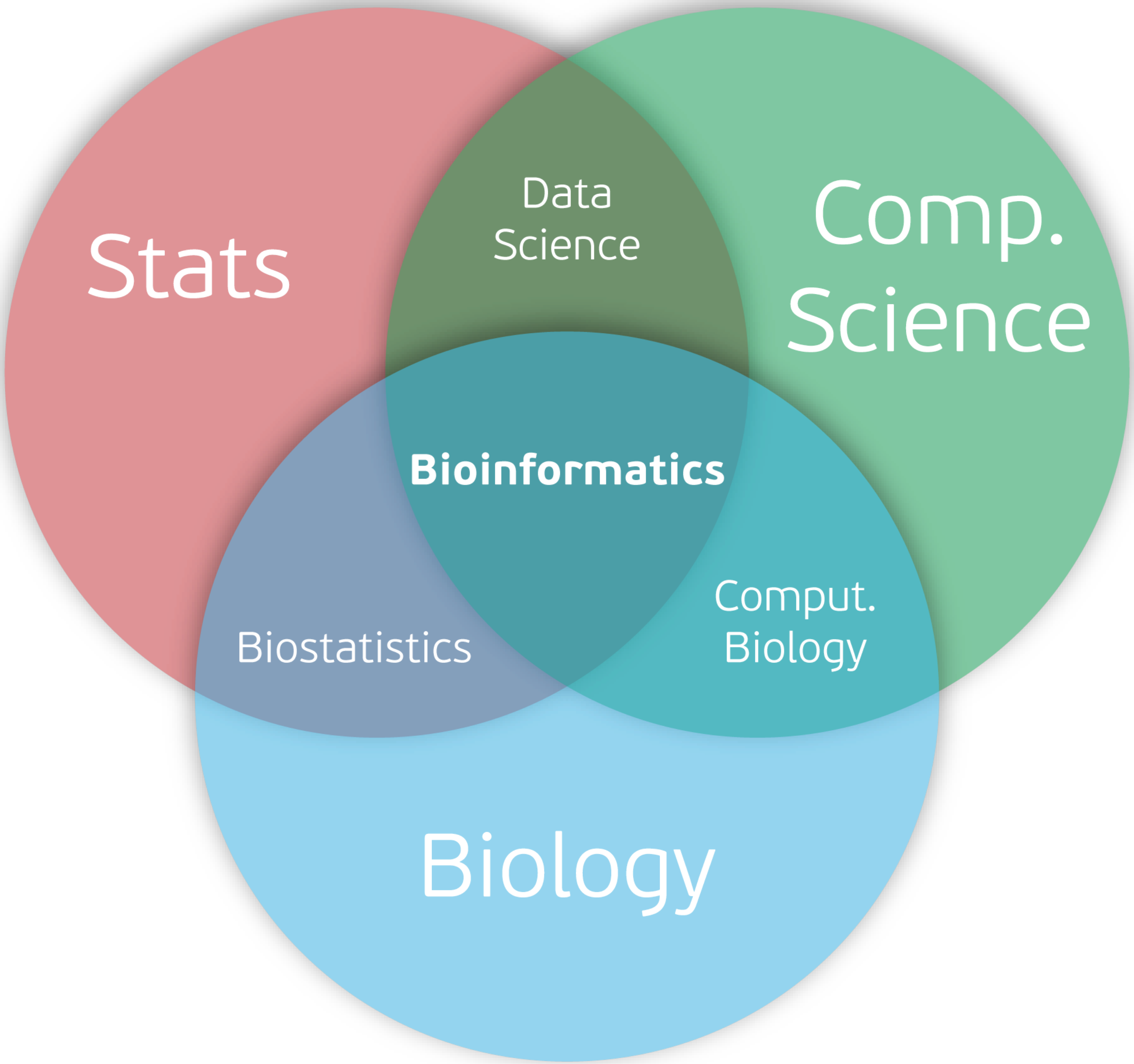
Increases in data requires advanced analyses (MiSeq)

- 15,000,000,000 ATGC's generated per sequencing run
- 40,000 - 150,000 words in a novel
- average word length in English is 4.79
- one sequencing run would generate **32,963** novels with 95,000 words each

Increases in data requires advanced analyses (Nextseq 2000)

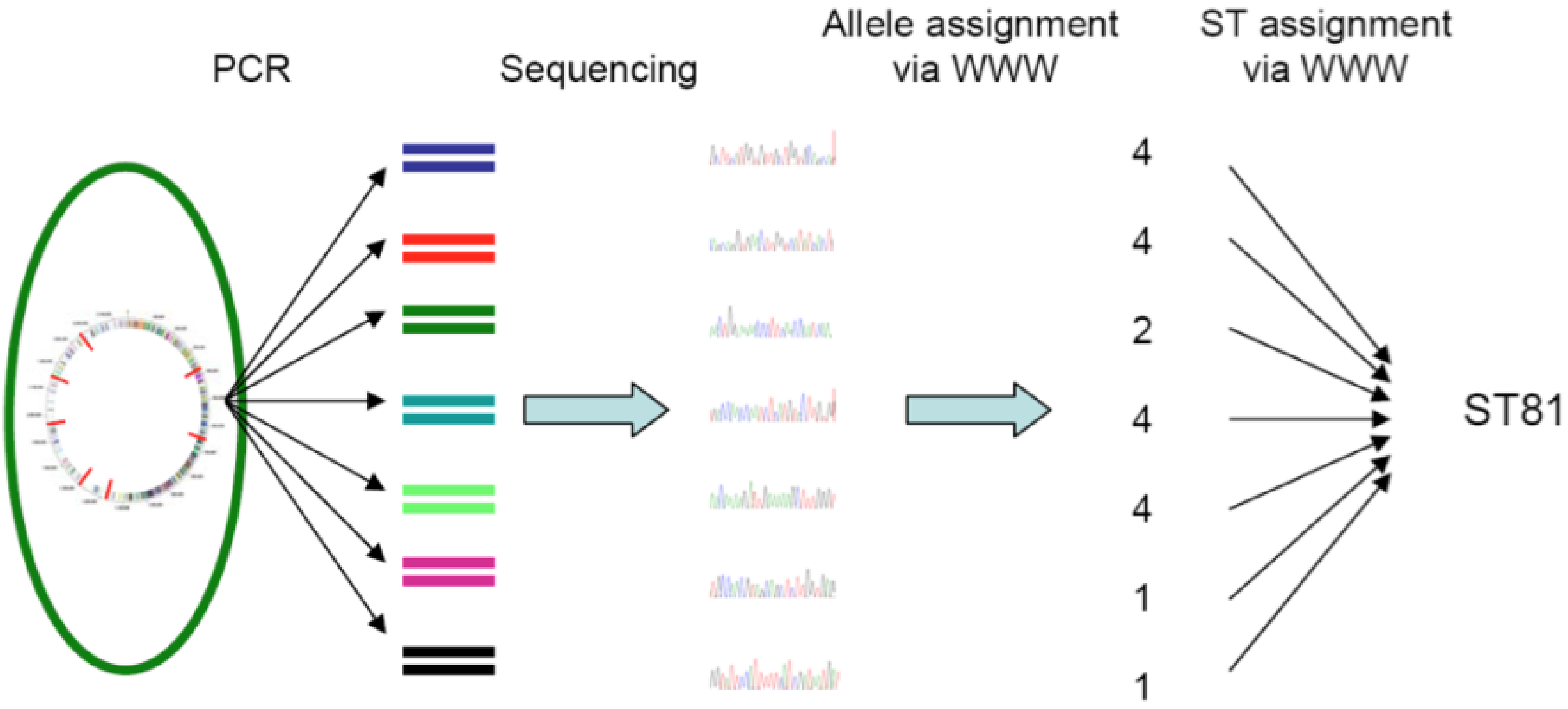
- 360,000,000,000 ATGC's generated per sequencing run
- 40,000 - 150,000 words in a novel
- average word length in English is 4.79
- one sequencing run would generate **791,121** novels with 95,000 words each



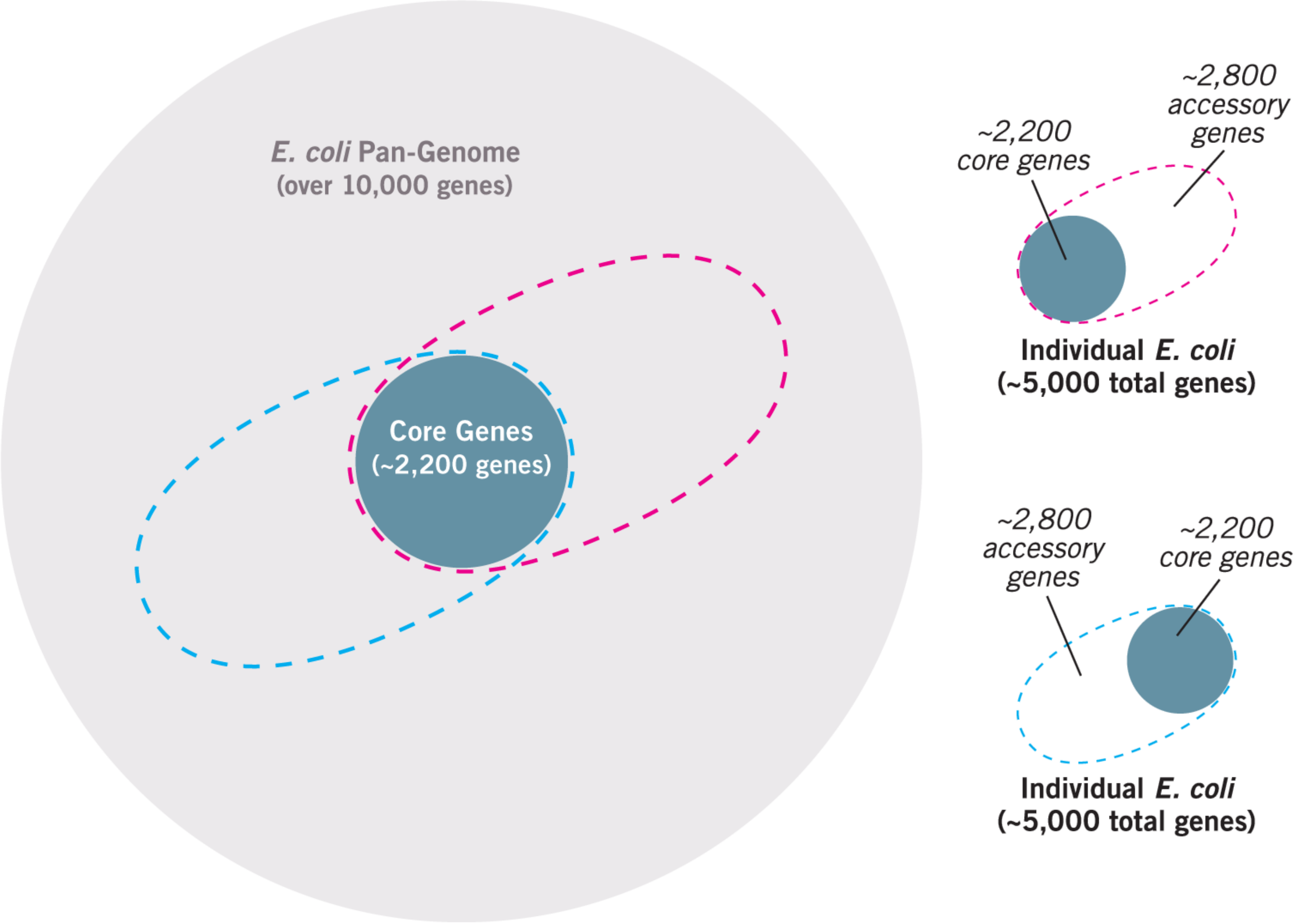
Stats
Data Science
Comp. Science
Bioinformatics
Biostatistics
Comput. Biology
Biology

Application of Next Generation Sequencing

Bacterial Genomic Classification - Multi Locus Sequence Typing



Bacterial Genetic Makeup



E. coli Pan-Genome
(over 10,000 genes)

Core Genes
(~2,200 genes)

~2,200
core genes

~2,800
accessory
genes

Individual *E. coli*
(~5,000 total genes)

~2,800
accessory
genes

~2,200
core genes

Individual *E. coli*
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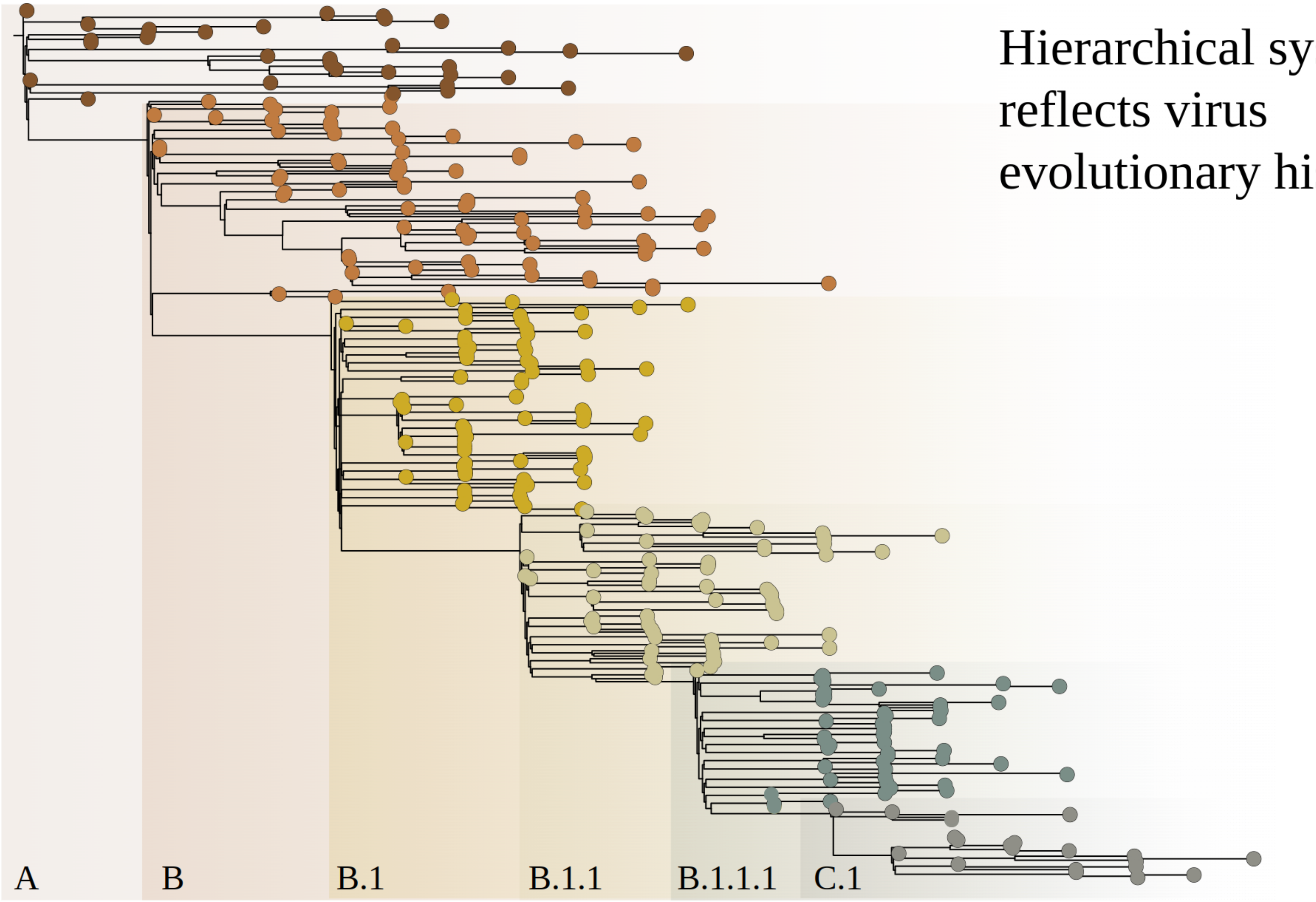
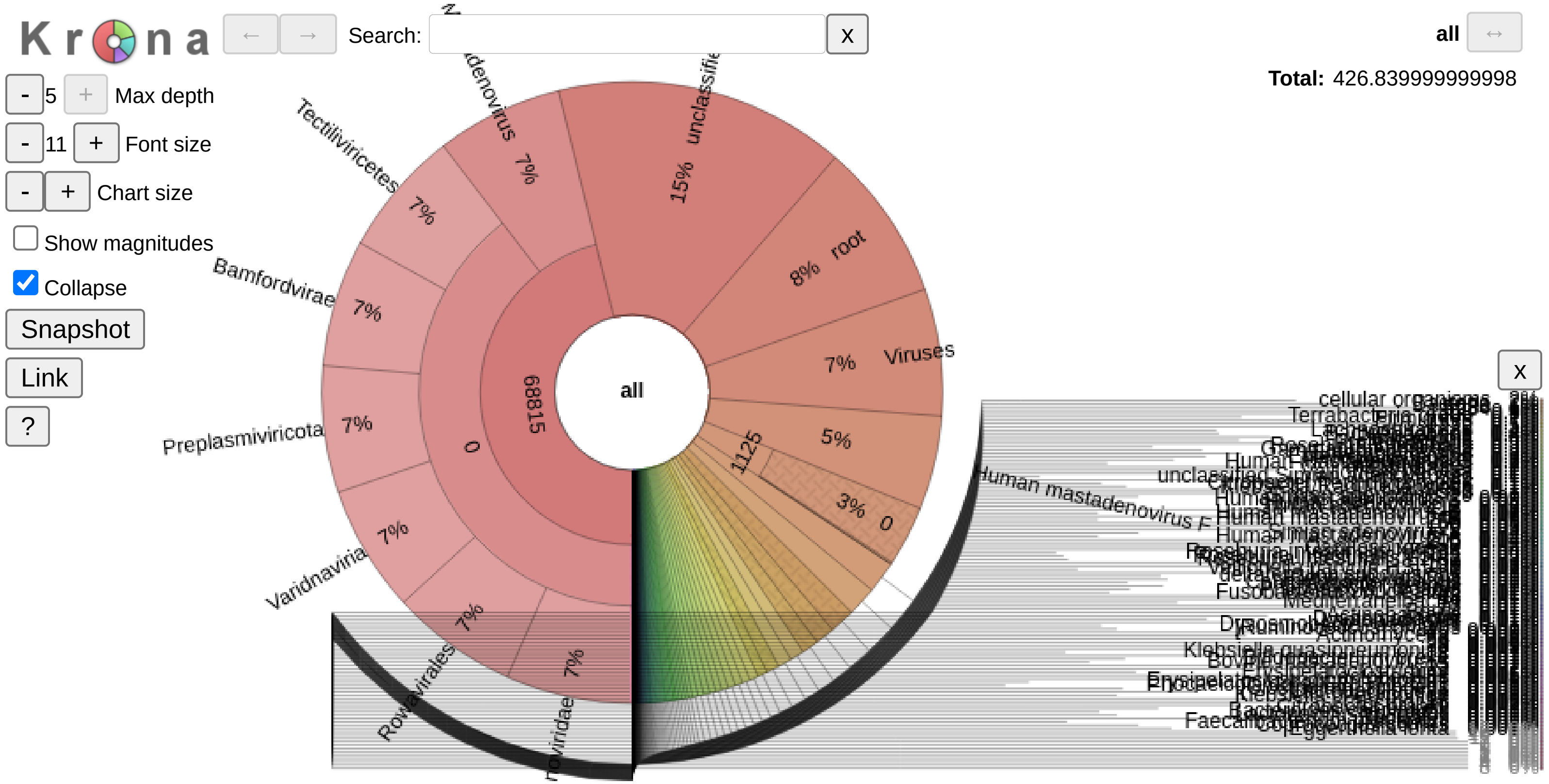
Viral Genomic Classification



Hierarchical system reflects virus evolutionary history

https://www.pango.network/

Metagenomic Classification



Max depth: 5

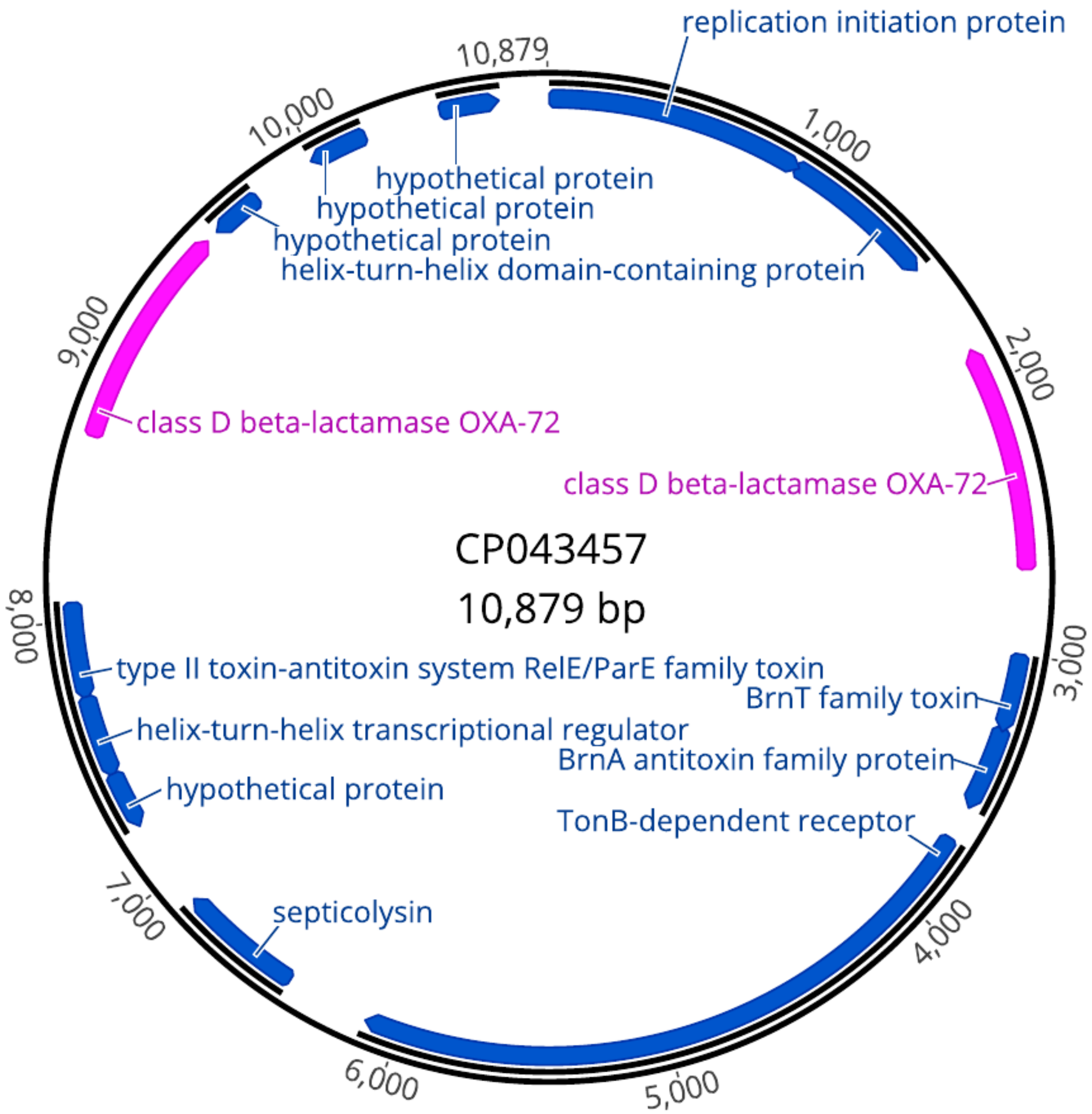
Font size: 11

☐ Show magnitudes

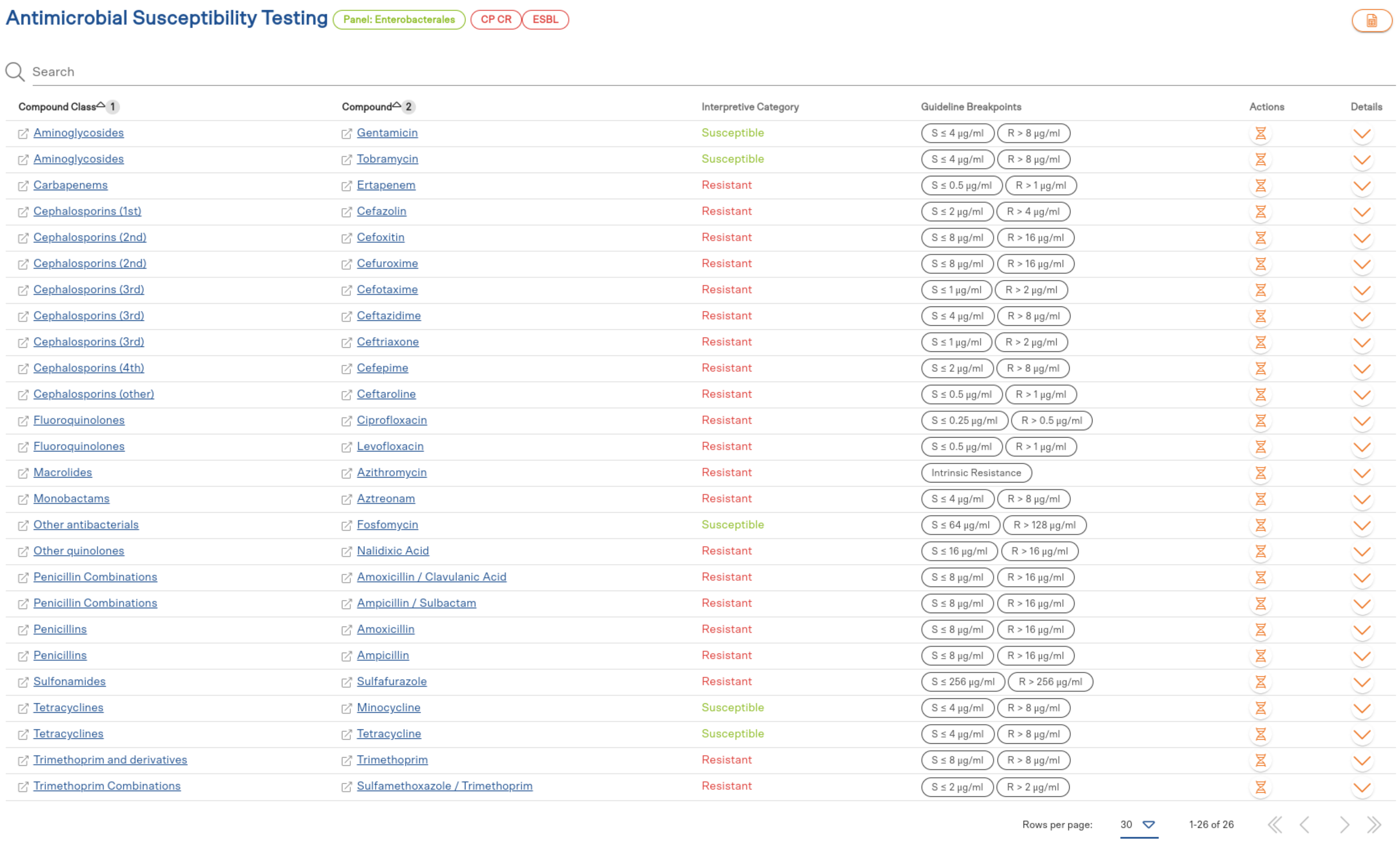
☑ Collapse

Total: 426.839999999998

Gene Identification



Phenotypic Prediction - Antimicrobial Susceptibility Test



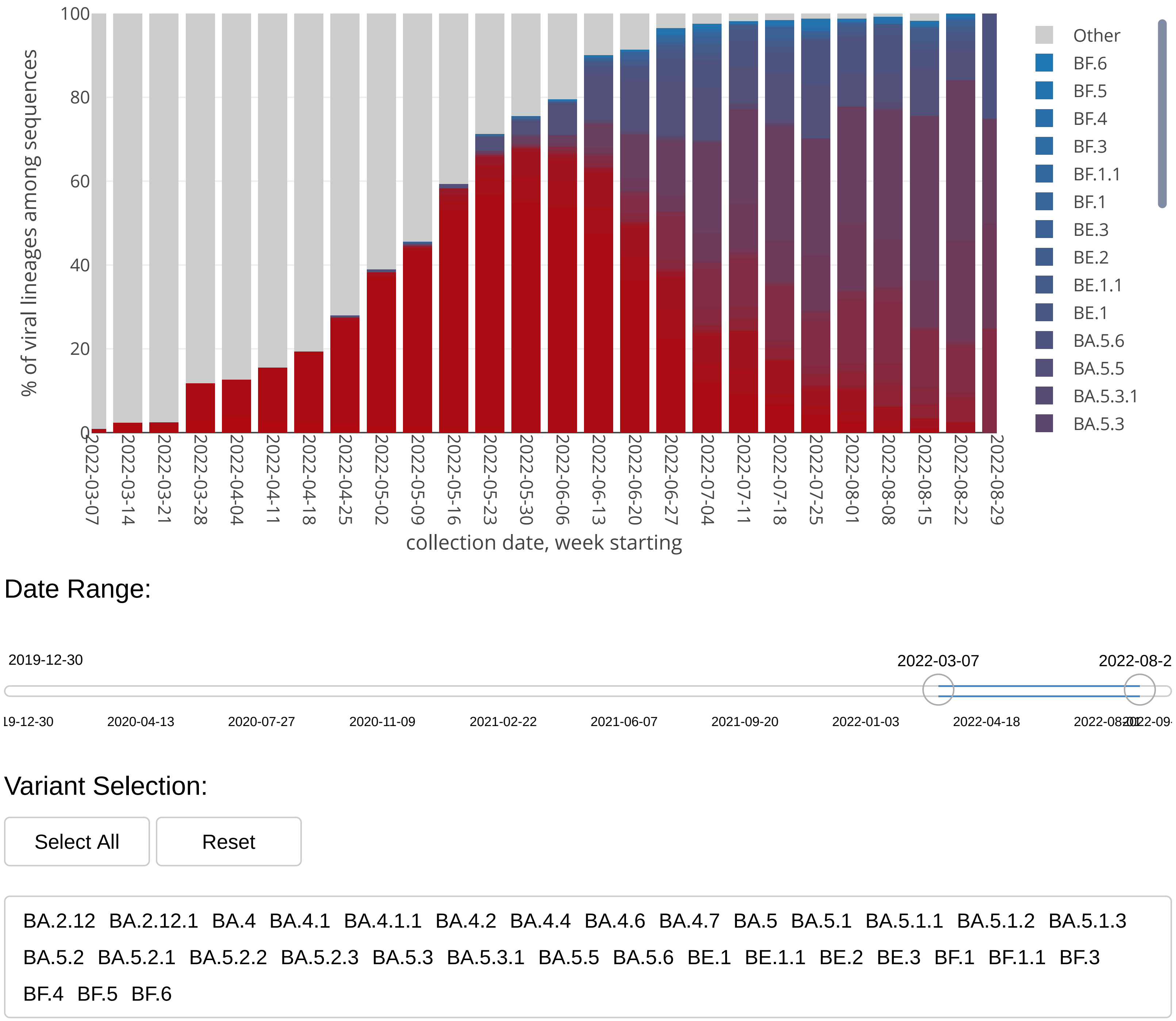
Antimicrobial Susceptibility Testing

Compound Class ^ 1	Compound ^ 2	Interpretive Category	Guideline Breakpoints	Actions	Details
Aminoglycosides	Gentamicin	Susceptible	S ≤ 4 µg/ml R > 8 µg/ml	⌛	∨
Aminoglycosides	Tobramycin	Susceptible	S ≤ 4 µg/ml R > 8 µg/ml	⌛	∨
Carbapenems	Ertapenem	Resistant	S ≤ 0.5 µg/ml R > 1 µg/ml	⌛	∨
Cephalosporins (1st)	Cefazolin	Resistant	S ≤ 2 µg/ml R > 4 µg/ml	⌛	∨
Cephalosporins (2nd)	Cefoxitin	Resistant	S ≤ 8 µg/ml R > 16 µg/ml	⌛	∨
Cephalosporins (2nd)	Cefuroxime	Resistant	S ≤ 8 µg/ml R > 16 µg/ml	⌛	∨
Cephalosporins (3rd)	Cefotaxime	Resistant	S ≤ 1 µg/ml R > 2 µg/ml	⌛	∨
Cephalosporins (3rd)	Ceftazidime	Resistant	S ≤ 4 µg/ml R > 8 µg/ml	⌛	∨
Cephalosporins (3rd)	Ceftriaxone	Resistant	S ≤ 1 µg/ml R > 2 µg/ml	⌛	∨
Cephalosporins (4th)	Cefepime	Resistant	S ≤ 2 µg/ml R > 8 µg/ml	⌛	∨
Cephalosporins (other)	Ceftaroline	Resistant	S ≤ 0.5 µg/ml R > 1 µg/ml	⌛	∨
Fluoroquinolones	Ciprofloxacin	Resistant	S ≤ 0.25 µg/ml R > 0.5 µg/ml	⌛	∨
Fluoroquinolones	Levofloxacin	Resistant	S ≤ 0.5 µg/ml R > 1 µg/ml	⌛	∨
Macrolides	Azithromycin	Resistant	Intrinsic Resistance	⌛	∨
Monobactams	Aztreonam	Resistant	S ≤ 4 µg/ml R > 8 µg/ml	⌛	∨
Other antibacterials	Fosfomycin	Susceptible	S ≤ 64 µg/ml R > 128 µg/ml	⌛	∨
Other quinolones	Nalidixic Acid	Resistant	S ≤ 16 µg/ml R > 16 µg/ml	⌛	∨
Penicillin Combinations	Amoxicillin / Clavulanic Acid	Resistant	S ≤ 8 µg/ml R > 16 µg/ml	⌛	∨
Penicillin Combinations	Ampicillin / Sulbactam	Resistant	S ≤ 8 µg/ml R > 16 µg/ml	⌛	∨
Penicillins	Amoxicillin	Resistant	S ≤ 8 µg/ml R > 16 µg/ml	⌛	∨
Penicillins	Ampicillin	Resistant	S ≤ 8 µg/ml R > 16 µg/ml	⌛	∨
Sulfonamides	Sulfafurazole	Resistant	S ≤ 256 µg/ml R > 256 µg/ml	⌛	∨
Tetracyclines	Minocycline	Susceptible	S ≤ 4 µg/ml R > 8 µg/ml	⌛	∨
Tetracyclines	Tetracycline	Susceptible	S ≤ 4 µg/ml R > 8 µg/ml	⌛	∨
Trimethoprim and derivatives	Trimethoprim	Resistant	S ≤ 8 µg/ml R > 8 µg/ml	⌛	∨
Trimethoprim Combinations	Sulfamethoxazole / Trimethoprim	Resistant	S ≤ 2 µg/ml R > 2 µg/ml	⌛	∨

ares genetics

Next Generation Sequencing in Public Health

Pathogen Genomic Surveillance

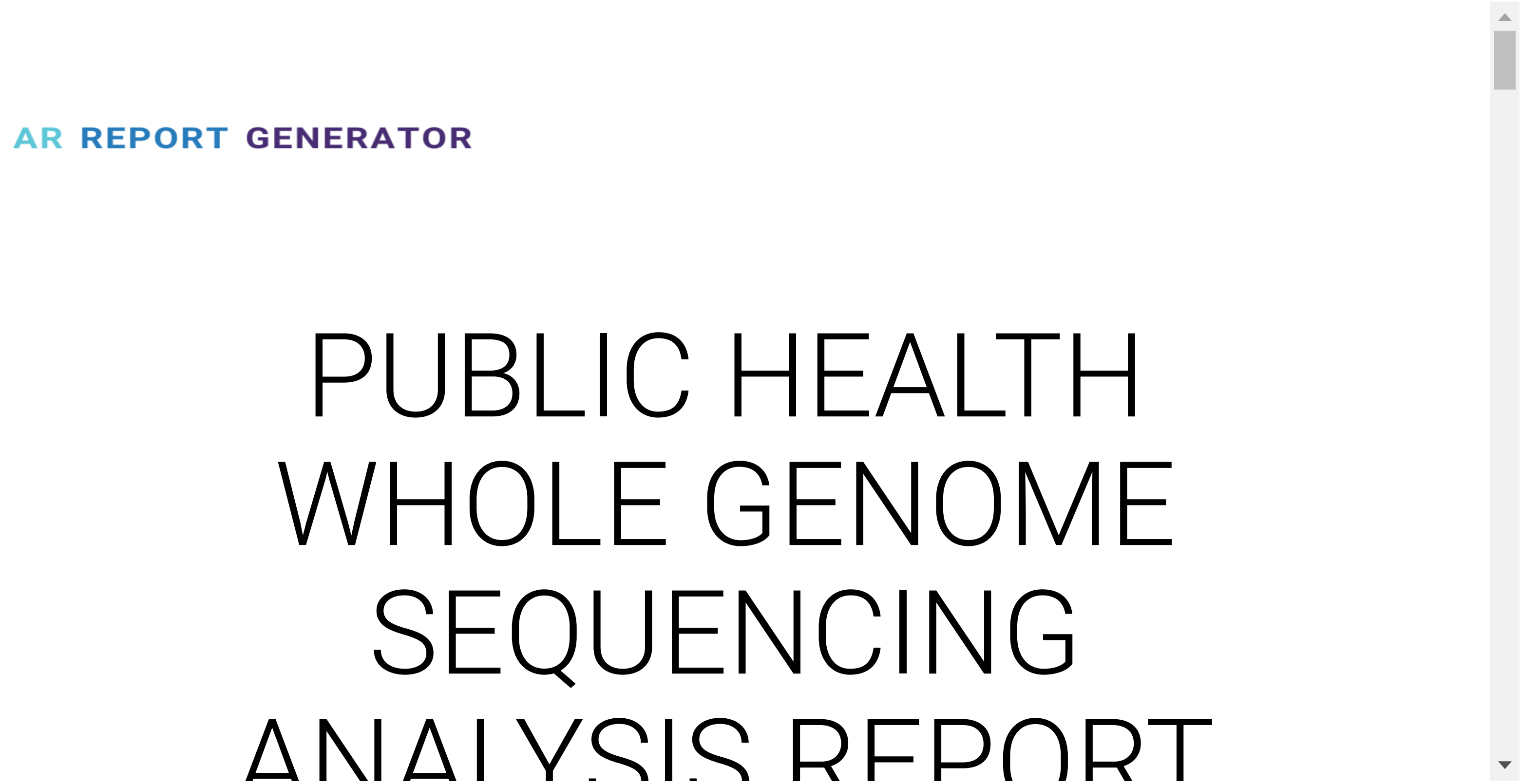


Date Range:

Variant Selection:

BA.2.12 BA.2.12.1 BA.4 BA.4.1 BA.4.1.1 BA.4.2 BA.4.4 BA.4.6 BA.4.7 BA.5 BA.5.1 BA.5.1.1 BA.5.1.2 BA.5.1.3 BA.5.2 BA.5.2.1 BA.5.2.2 BA.5.2.3 BA.5.3 BA.5.3.1 BA.5.5 BA.5.6 BE.1 BE.1.1 BE.2 BE.3 BF.1 BF.1.1 BF.3 BF.4 BF.5 BF.6

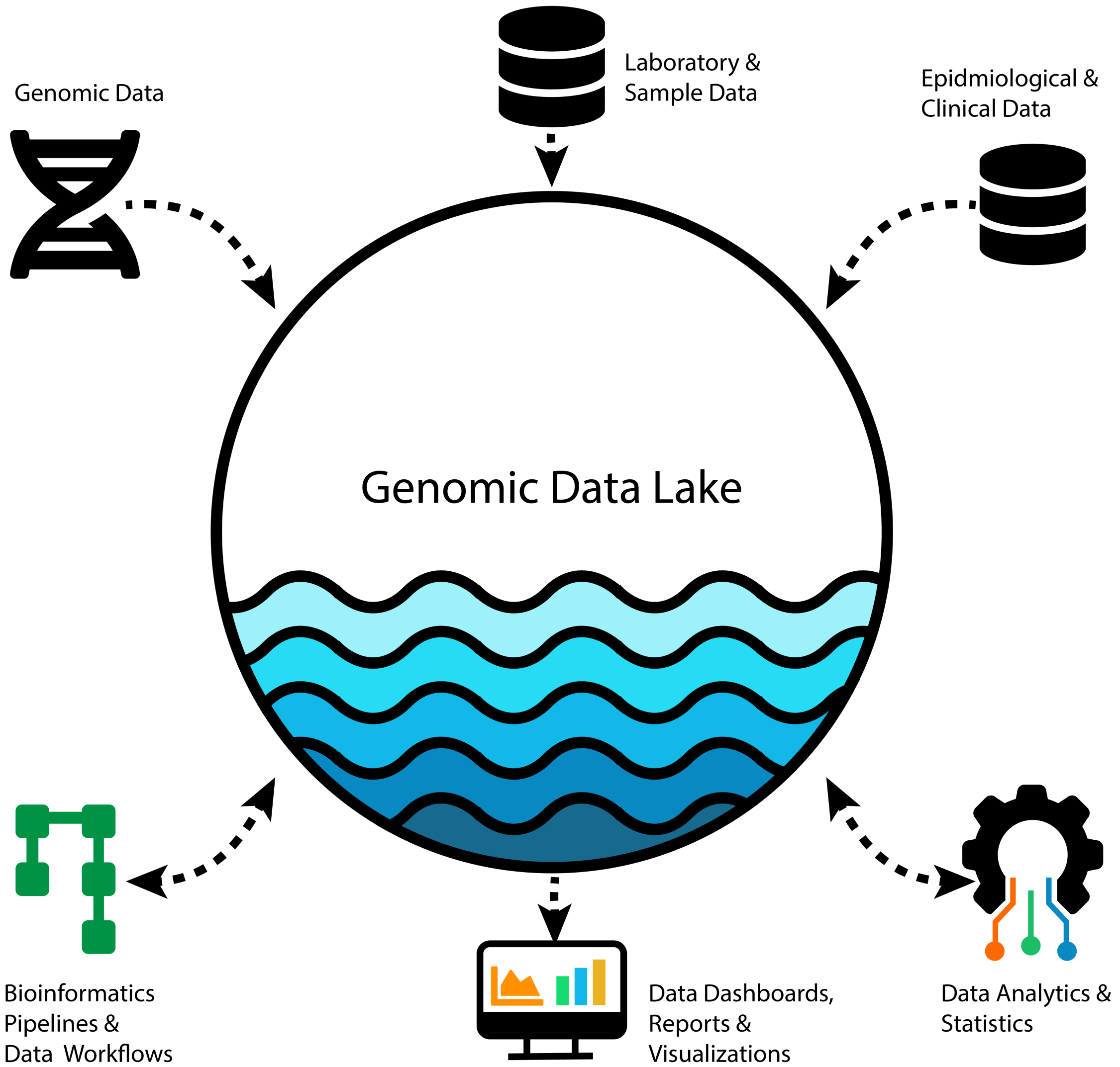
Outbreak Investigations



AR REPORT GENERATOR

PUBLIC HEALTH WHOLE GENOME SEQUENCING ANALYSIS REPORT

Managing a growing data infrastructure



Genomic Data

Laboratory &
Sample Data

Epidmiological &
Clinical Data

Genomic Data Lake

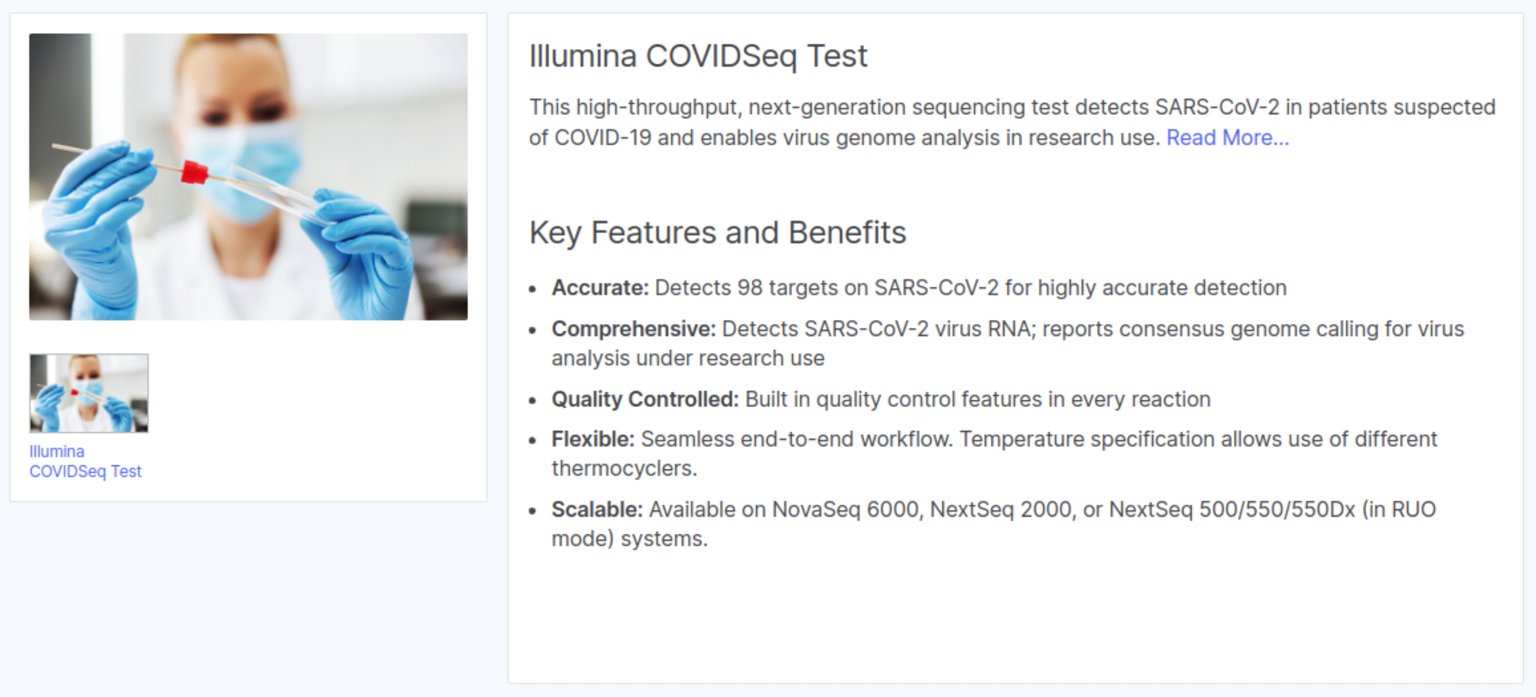
Bioinformatics
Pipelines &
Data Workflows

Data Dashboards,
Reports &
Visualizations

Data Analytics &
Statistics

Next Generation Sequencing and Diagnostic Testing

EUA Approved Illumina COVIDSeq Test



Illumina
COVIDSeq Test

Illumina COVIDSeq Test

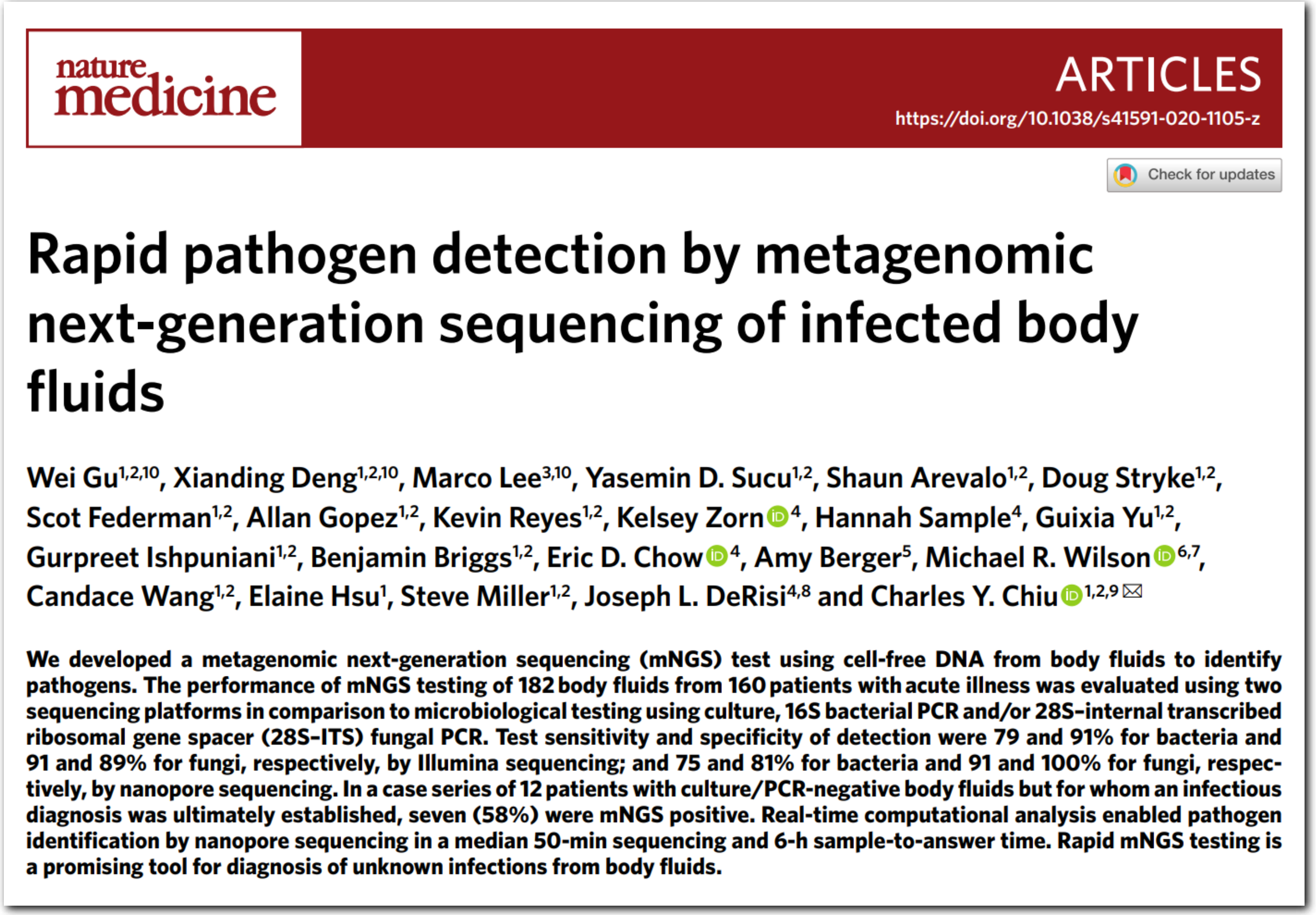
This high-throughput, next-generation sequencing test detects SARS-CoV-2 in patients suspected of COVID-19 and enables virus genome analysis in research use. Read More...

Key Features and Benefits

- **Accurate:** Detects 98 targets on SARS-CoV-2 for highly accurate detection
- **Comprehensive:** Detects SARS-CoV-2 virus RNA; reports consensus genome calling for virus analysis under research use
- **Quality Controlled:** Built in quality control features in every reaction
- **Flexible:** Seamless end-to-end workflow. Temperature specification allows use of different thermocyclers.
- **Scalable:** Available on NovaSeq 6000, NextSeq 2000, or NextSeq 500/550/550Dx (in RUO mode) systems.

Illumina COVIDSeq

Metagenomic Pathogen Detection



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ARTICLES

https://doi.org/10.1038/s41591-020-1105-z

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Gu et al. *Nat Med.* 2021

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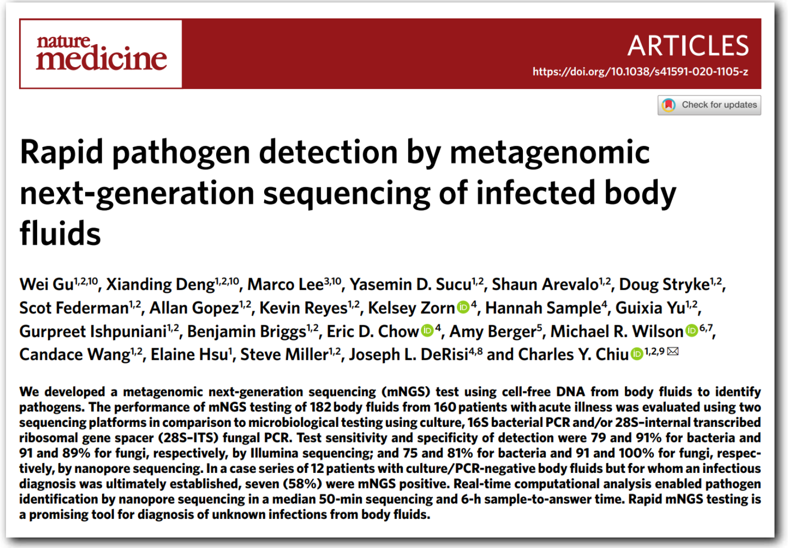

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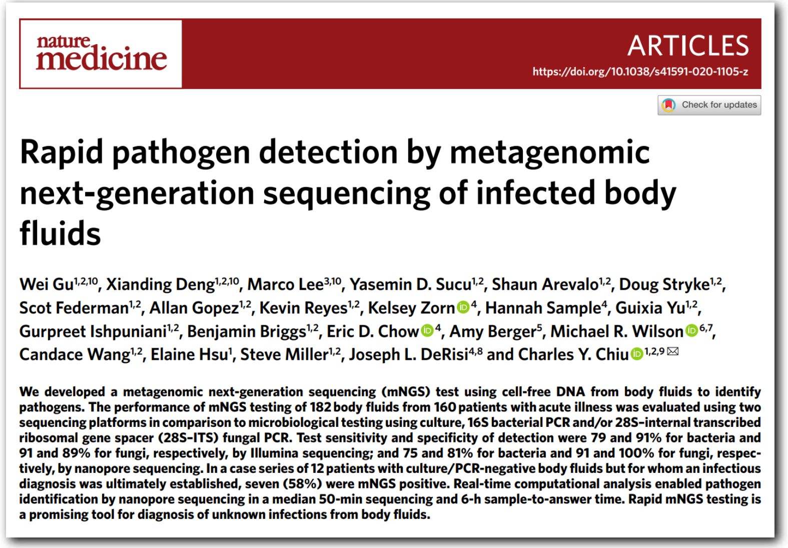
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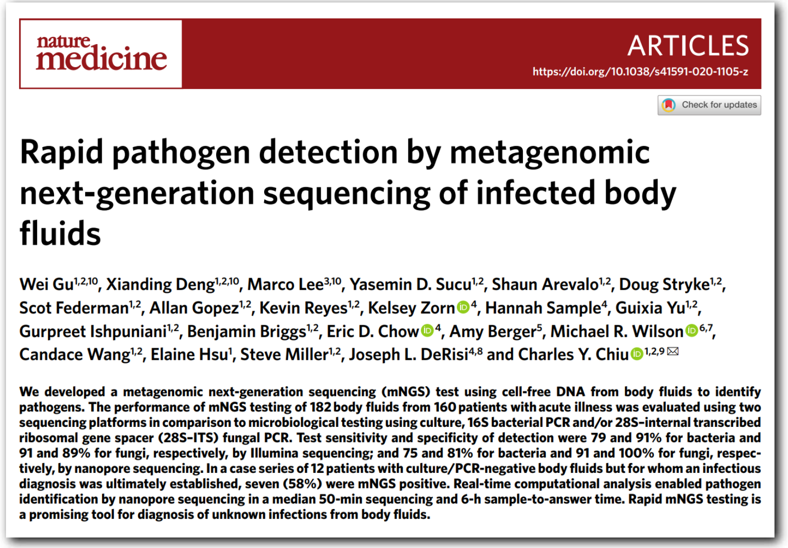

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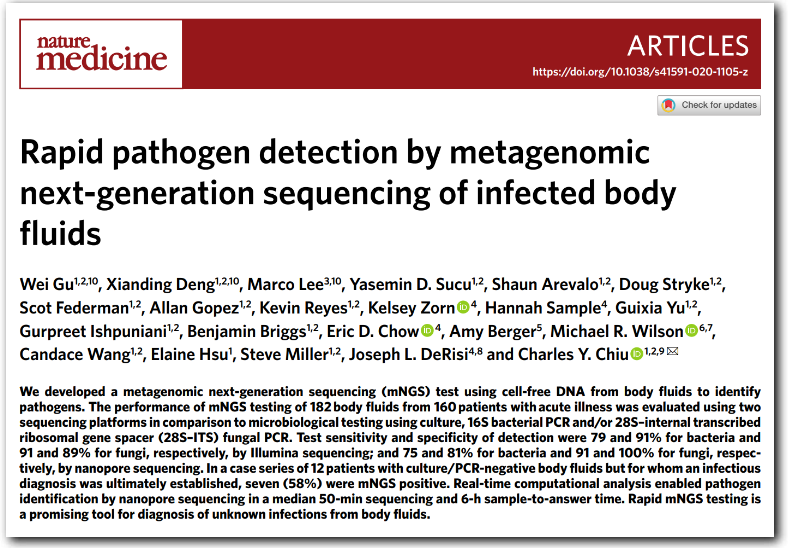

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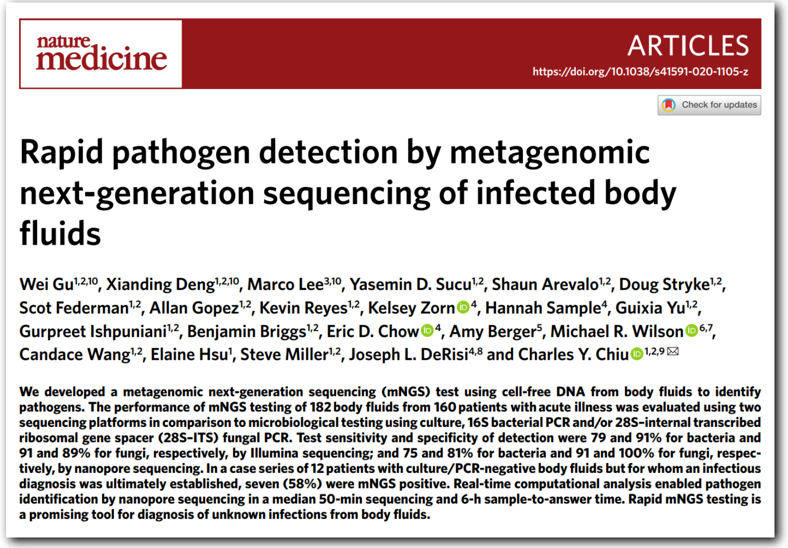

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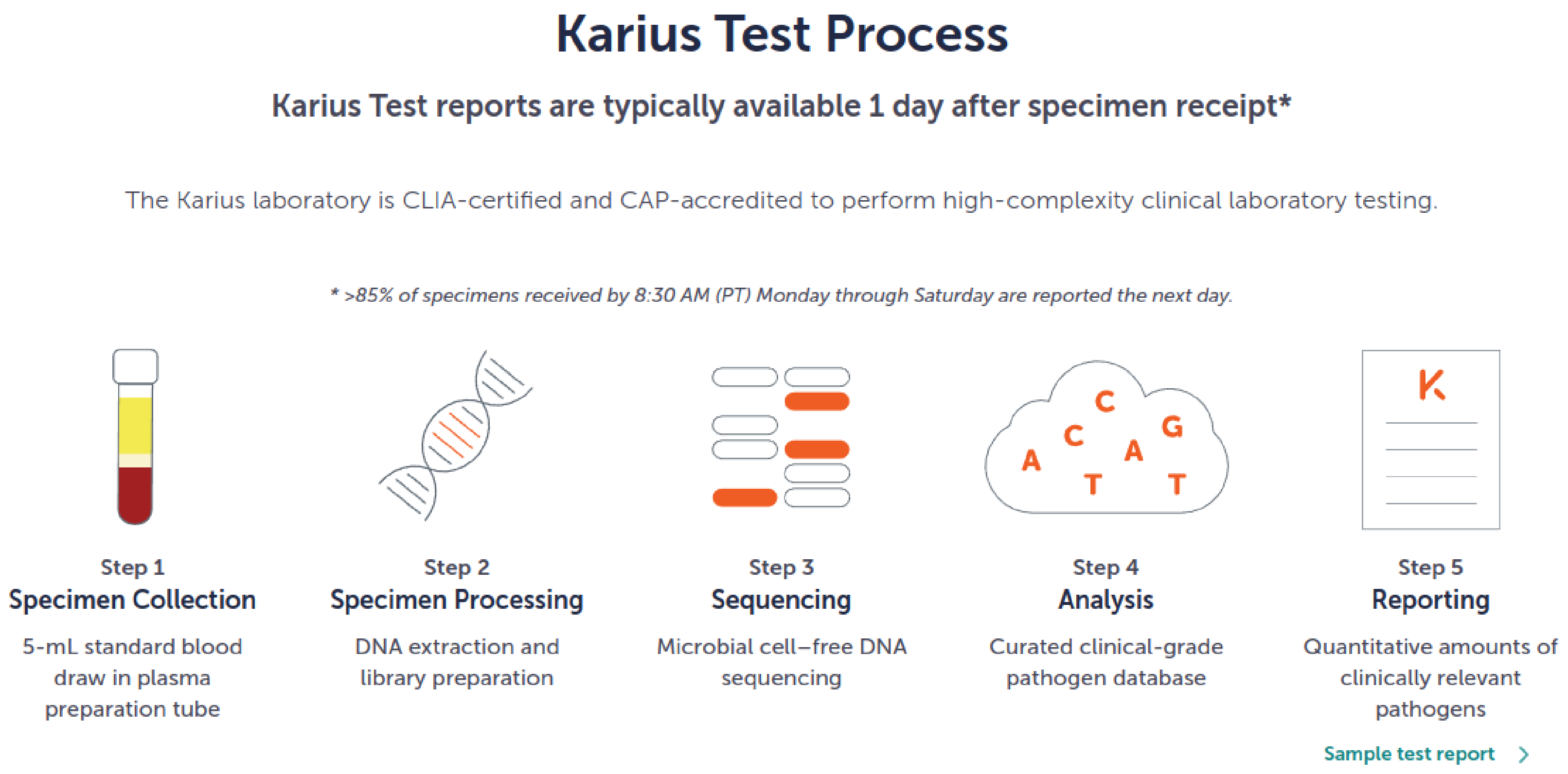
  - Bacteria: 75% Sensitivity, 81% Specificity
  - Fungi: 91% Sensitivity, 100% Specificity
- Case Series of 12 culture/PCR-negative patients with infectious diagnosis; 7 were mNGS positive
- Real time analysis enabled by Oxford Nanopore enabled pathogen identification in median of 50 minutes and a 6 hour sample to answer time

Gu et al. *Nat Med.* 2021

Metagenomic Pathogen Detection

KARIUS
clarity at speed®



Karius Test Process

Karius Test reports are typically available 1 day after specimen receipt*

The Karius laboratory is CLIA-certified and CAP-accredited to perform high-complexity clinical laboratory testing.

** >85% of specimens received by 8:30 AM (PT) Monday through Saturday are reported the next day.*

Step 1
Specimen Collection
5-mL standard blood draw in plasma preparation tube

Step 2
Specimen Processing
DNA extraction and library preparation

Step 3
Sequencing
Microbial cell–free DNA sequencing

Step 4
Analysis
Curated clinical-grade pathogen database

Step 5
Reporting
Quantitative amounts of clinically relevant pathogens

Sample test report ›

https://kariusdx.com/

Metagenomic Pathogen Detection

idbydna

User-friendly and Intuitive

Designed by the lab for the lab. Automated and intuitive graphical user interfaces allow any lab to leverage Precision Metagenomics without the learning curve.

Rapid Turnaround

Convert millions of DNA sequences into high-confidence results in minutes, powered by artificial intelligence and other optimized algorithms.

Actionable and Trusted Insight

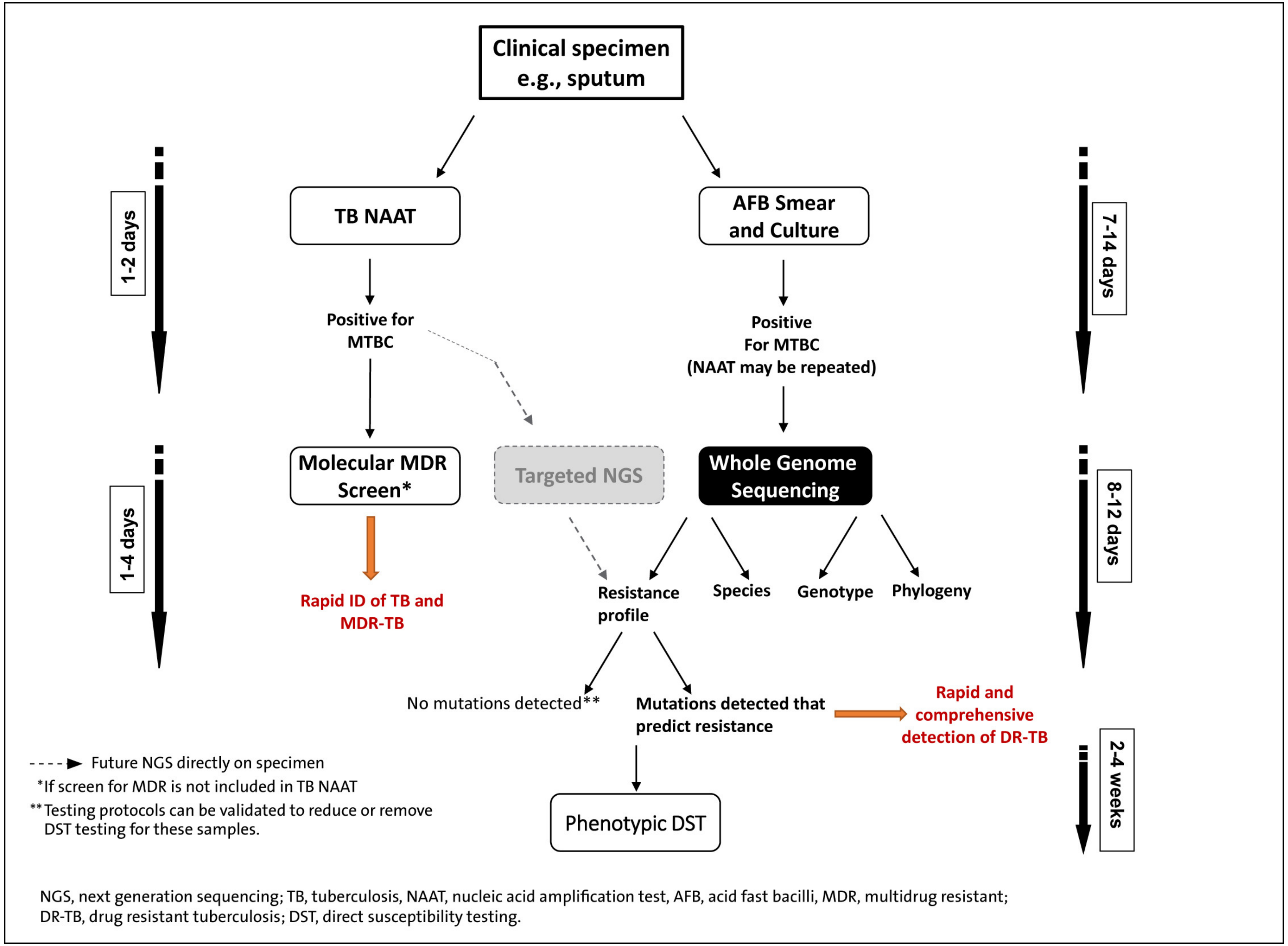
Actionable insights into thousands of pathogens with known links to disease, combined with predicted drug resistance markers, are provided in an easy to interpret report, powered by artificial intelligence.

Complete Pathogen and AMR Profiling

Detects 50,000+ viruses, bacteria, fungi and parasites, including 6,000+ known human pathogens. Profiles antimicrobial resistance for viruses, bacteria, and fungi at high-resolution.

https://idbydna.com/

Diagnosis of Antimicrobial Resistance in Tuberculosis



Clinical specimen e.g., sputum

1-2 days

TB NAAT

Positive for MTBC

Molecular MDR Screen*

1-4 days

Rapid ID of TB and MDR-TB

Targeted NGS

7-14 days

AFB Smear and Culture

Positive For MTBC (NAAT may be repeated)

Whole Genome Sequencing

8-12 days

Resistance profile

Species

Genotype

Phylogeny

No mutations detected**

Mutations detected that predict resistance

Rapid and comprehensive detection of DR-TB

2-4 weeks

Phenotypic DST

- - - -► Future NGS directly on specimen

*If screen for MDR is not included in TB NAAT

**Testing protocols can be validated to reduce or remove DST testing for these samples.

NGS, next generation sequencing; TB, tuberculosis, NAAT, nucleic acid amplification test, AFB, acid fast bacilli, MDR, multidrug resistant; DR-TB, drug resistant tuberculosis; DST, direct susceptibility testing.

Rowlinson and Musser *Clinical Microbiology Newsletter* 2022

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Reveal.JS

Gourmet Pop Rocks

Pastry Chef Attempts to Make Gourmet Pop Rocks | Gourmet Makes | Bon Appétit

Questions?