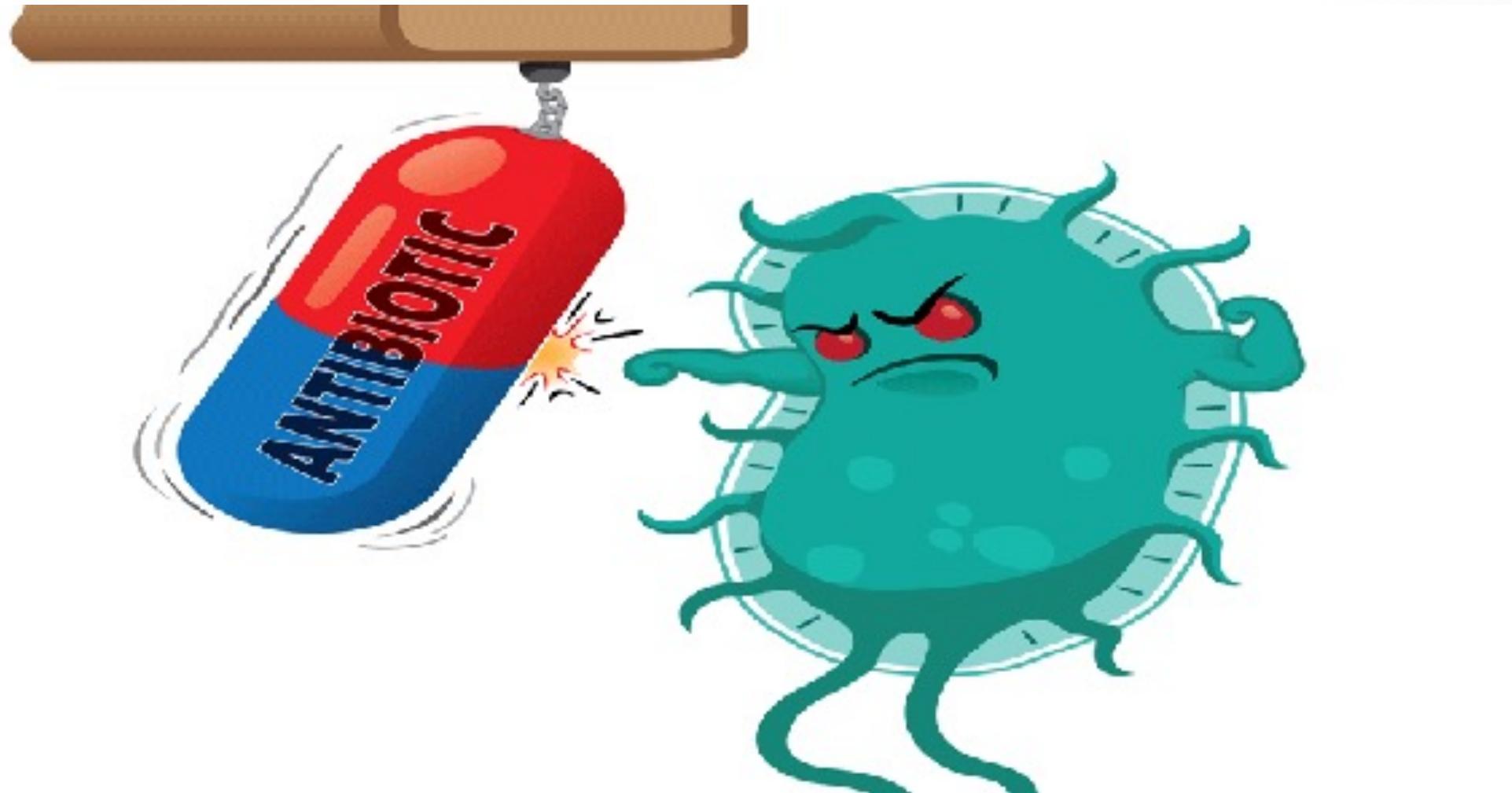


## 9. Resistome

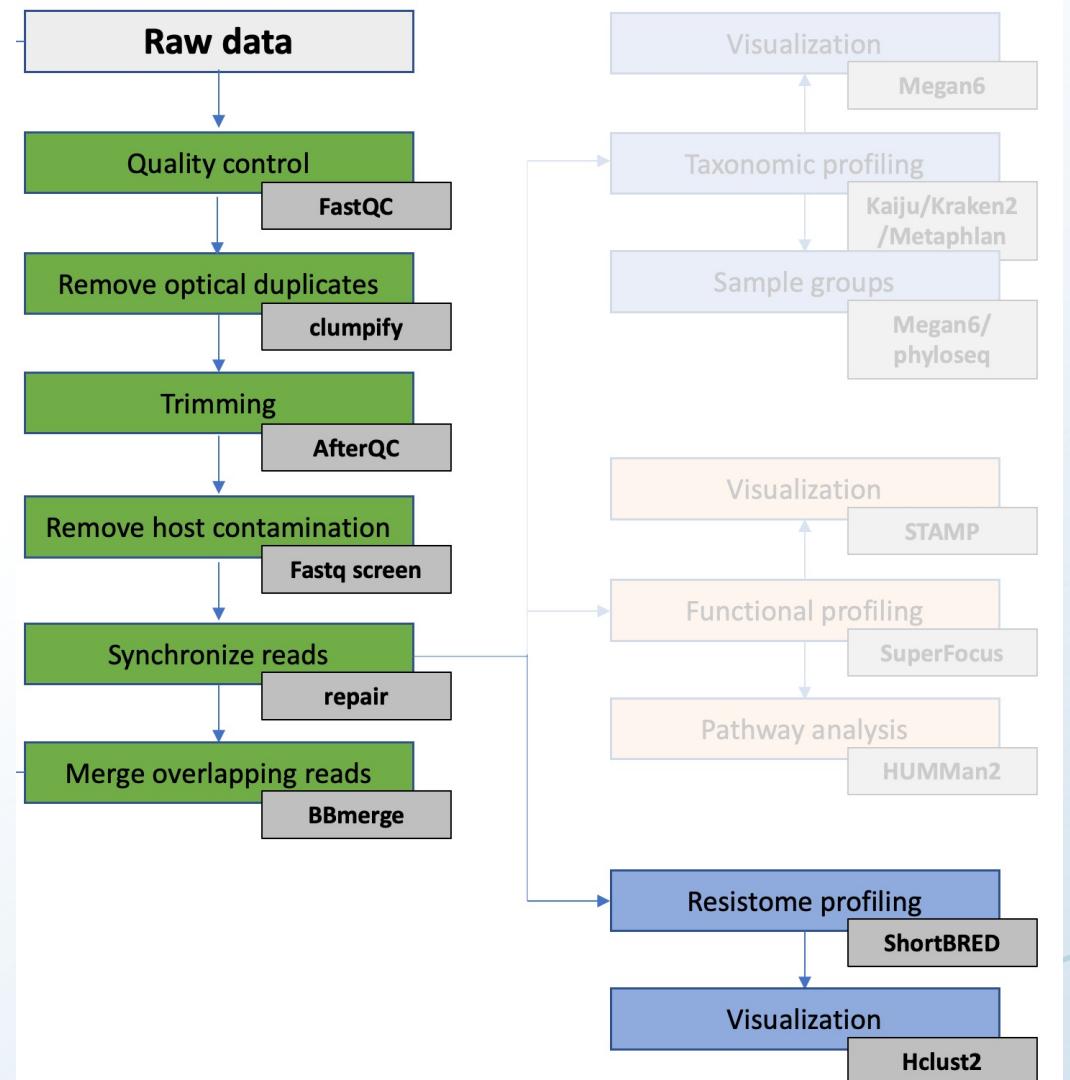


# Overview of this talk

Antimicrobial resistance

Human resistome

Shortbred



# **Antimicrobial resistance**

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Antimicrobial resistance (AMR) is the ability of a microorganism (e.g., a bacterium, a virus) to resist the action of an antimicrobial agent

AMR is a global concern because new resistance mechanisms are emerging and are spreading globally

Misuse and overuse of antimicrobials is accelerating this process

AMR-microbes are found in people, animals, food, and the environment and can spread between these



**World Health  
Organization**

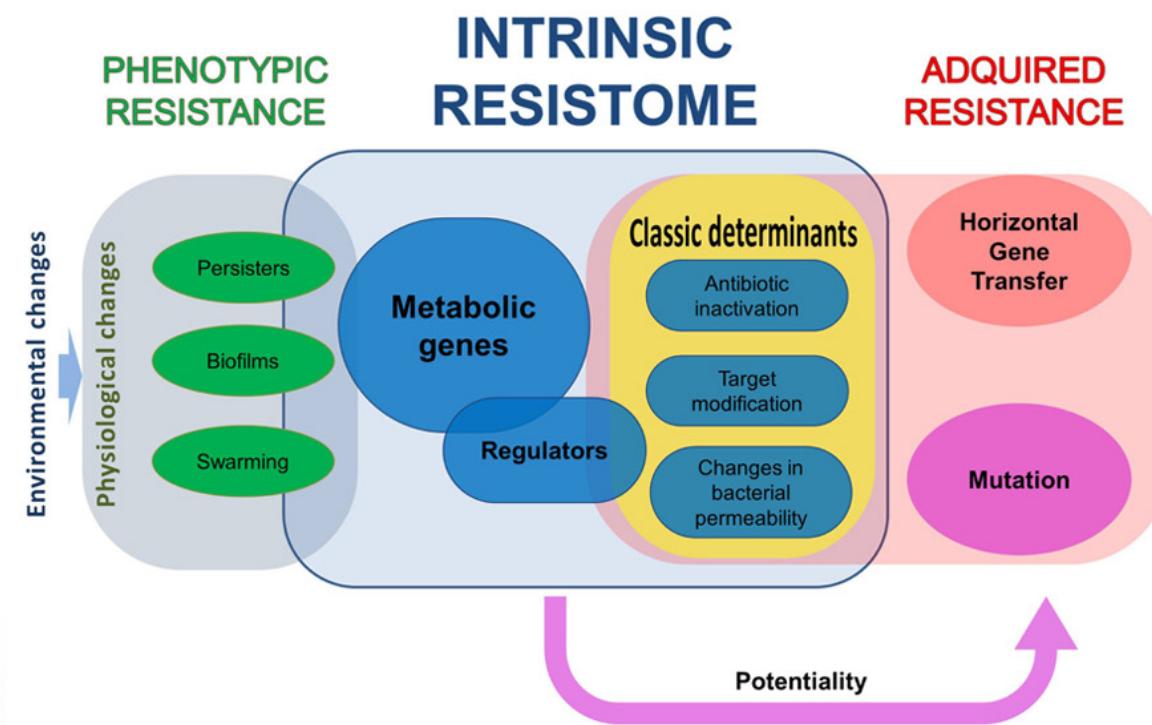
**The world is running out of  
antibiotics, WHO report confirms**

# Acquired, intrinsic and phenotypic antimicrobial resistance

Intrinsic = physiological trait; such as absence of a drug target

Acquired = heritable change in the DNA; such as mutations or acquiring ARGs

Phenotypic = non-inheritable resistance, such as growth in biofilms

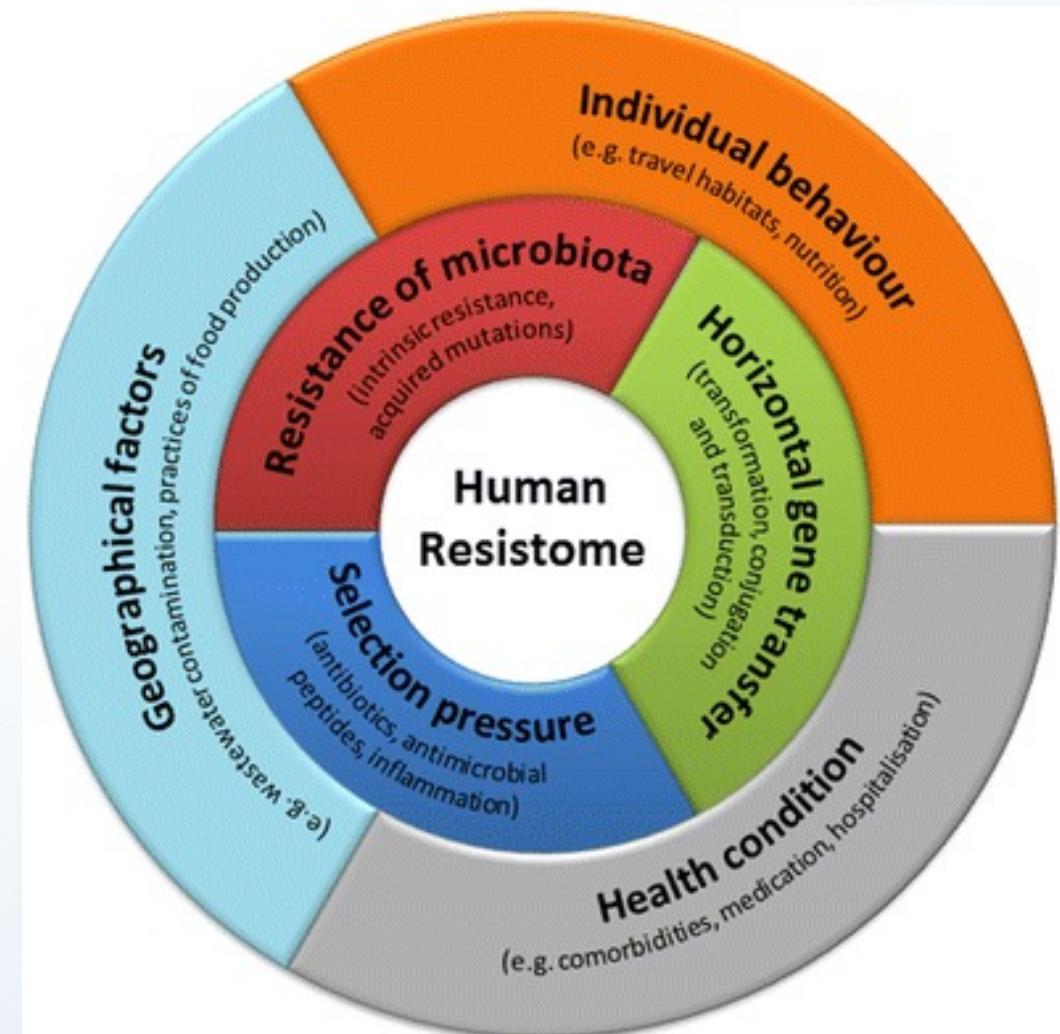


# The human (antibiotic) resistome – a dynamic entity

Resistome = Genes that may encode antimicrobial resistance in various microbial ecosystems

The human body harbours at various sites a complex microbial ecosystem and represents a vast reservoir for antimicrobial resistance genes (ARGs)

Many factors can potentially be involved in shaping the human resistome



# **How do we study the human resistome - Culture-dependent vs culture-independent methods**

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Culture-dependent underestimates species and ARG diversity

- Isolate and identify individual strains

- Only a minority of bacterial organisms can be cultured

Culture-independent methods (metagenomics) can potentially characterize the (complete) human resistome

- Lack of bias

- Less sensitive than targeted, culture-based methods

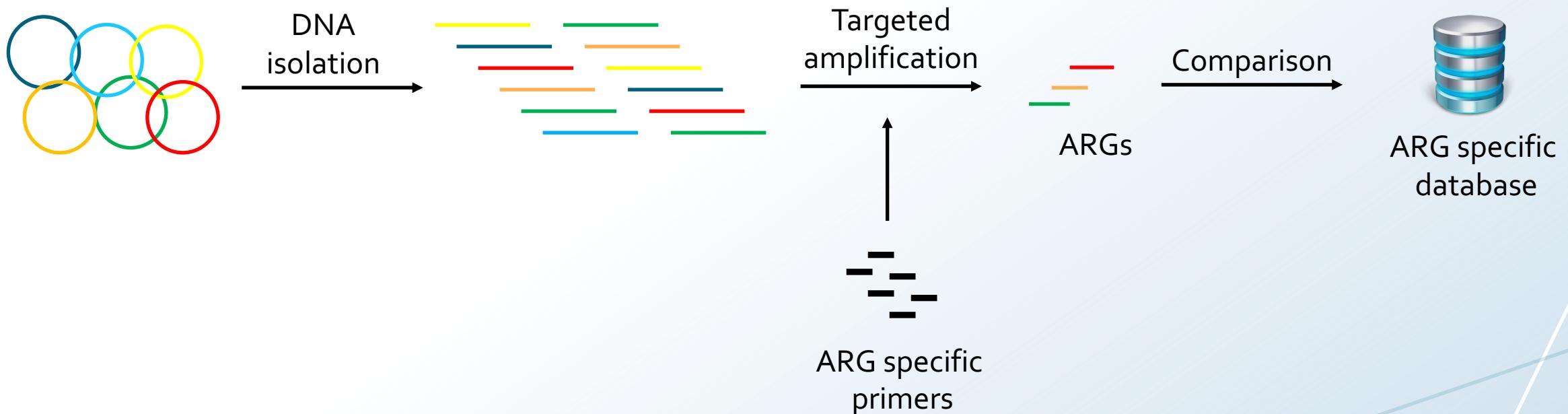
- Allows for a 'One Health' approach - considering human, animal and environmental AMR reservoirs

# Targeted (PCR-based) metagenomics for resistome profiling

PCR amplification of ARGs of interest

Limited to track known ARGs

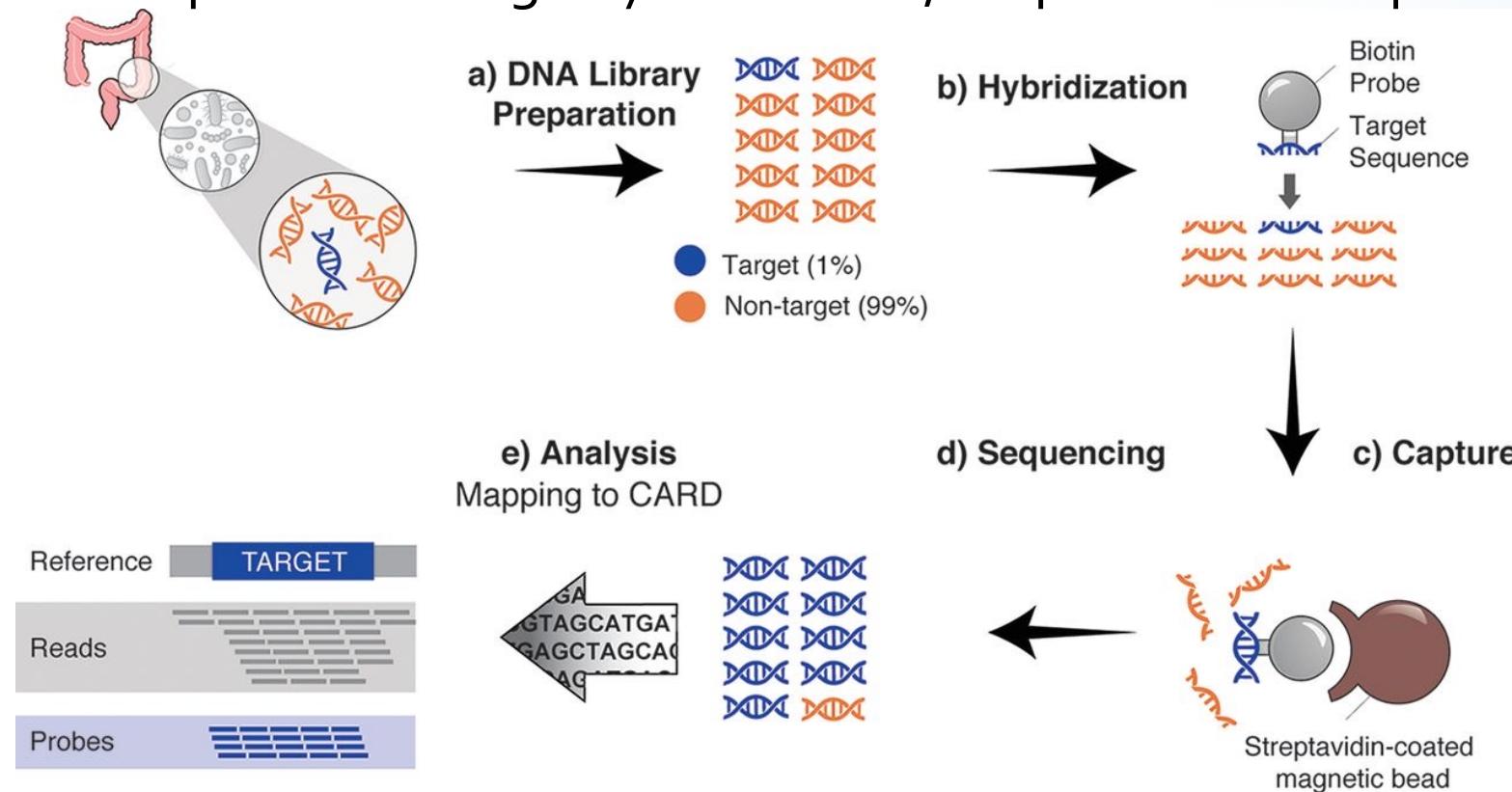
Do not give any information regarding the bacterial host of the ARGs



# Target capture metagenomics for resistome profiling

37,826 probes to specifically target over 2,000 ARGs in clinically relevant bacteria

Target DNA are captured through hybridization, amplified and sequenced

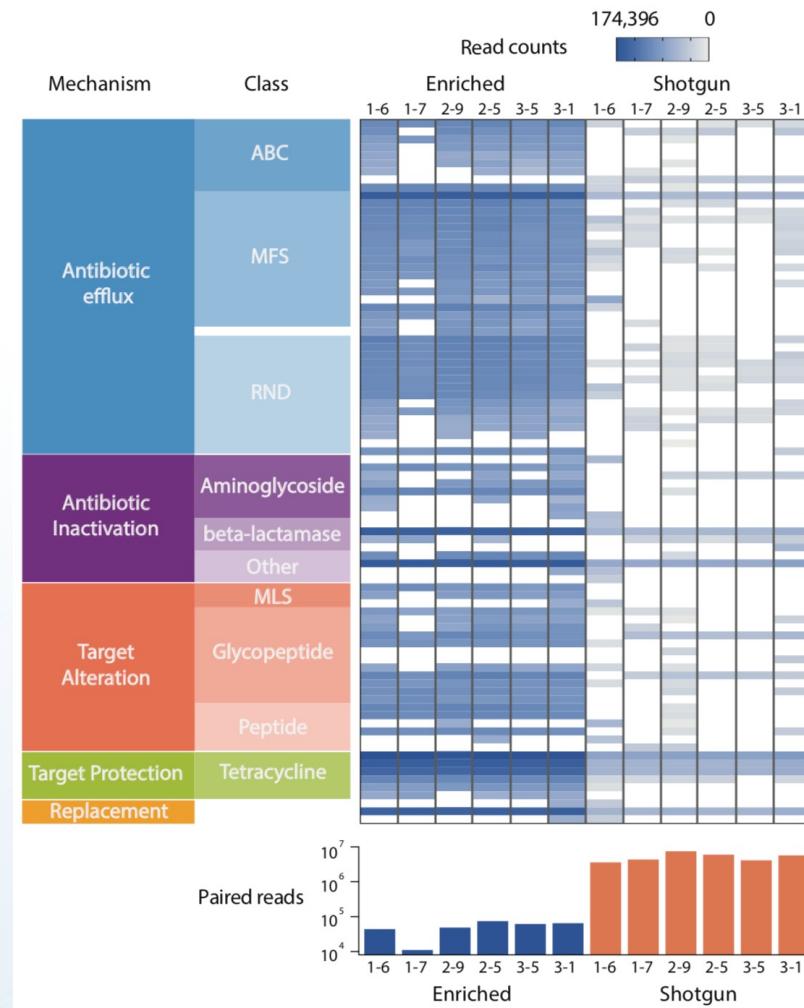


# Target capture metagenomics for resistome profiling

Identified additional resistance gene sequences from human gut microbiome samples that sequencing alone was not able to detect.

Genes encoding antibiotic resistance represent less than 0.1% of the metagenome

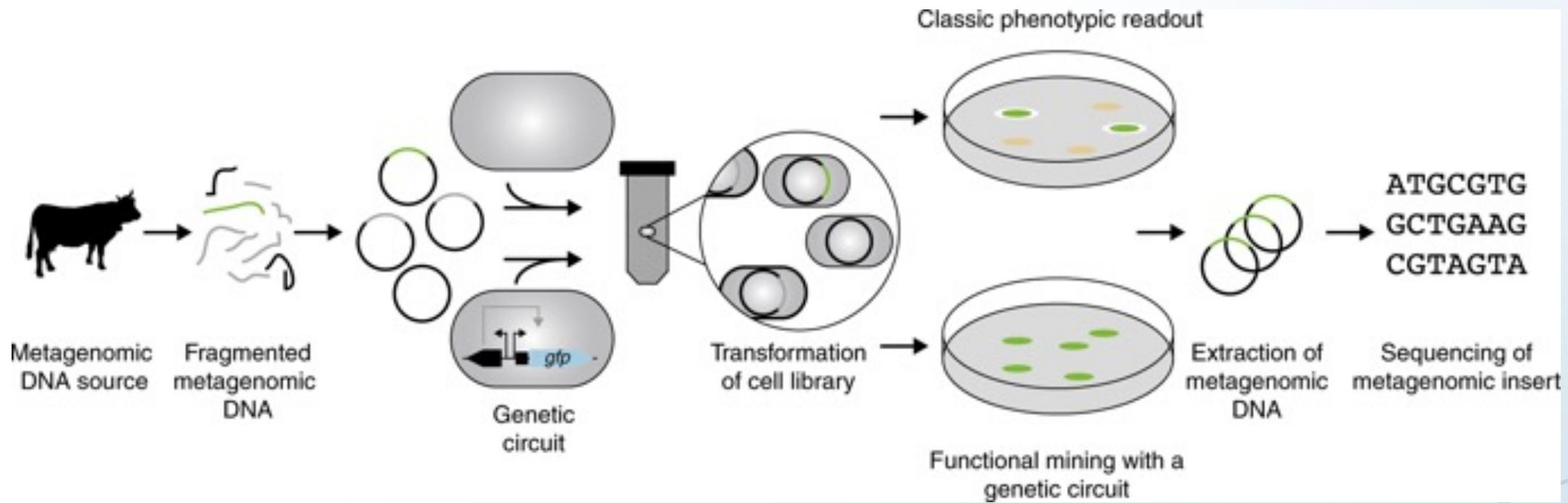
This method to capture the resistome enables a sensitive means of gene detection



# Functional metagenomics for resistome profiling

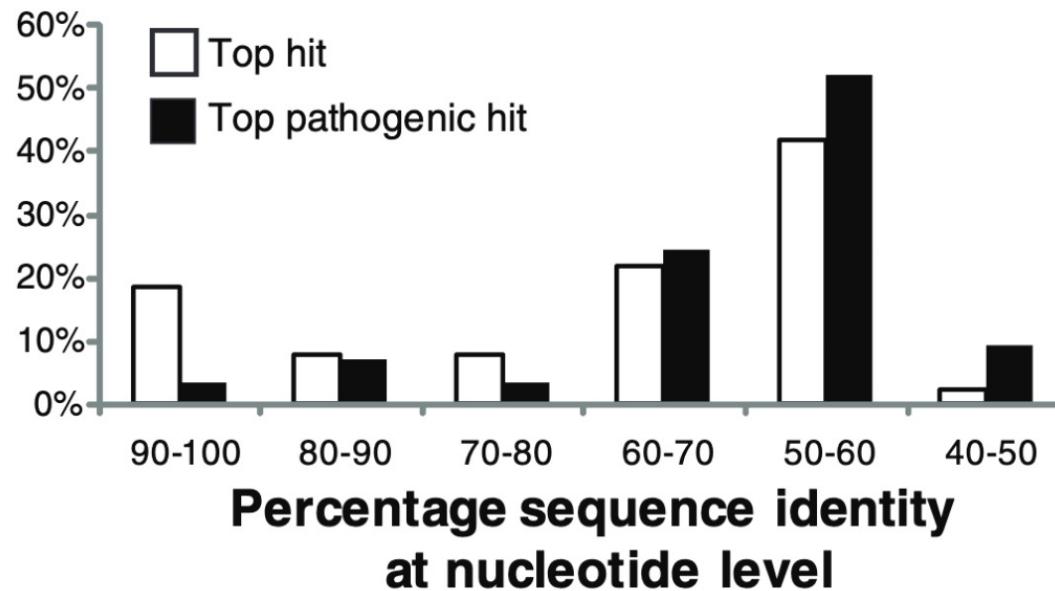
Does not require any prior knowledge about the resistance genes

Only metagenomics method available which allows the isolation of completely novel AGRs on a large scale



# Functional metagenomics for resistome profiling

Identification of ARGs with low similarity to known ARGs from pathogenic isolates



Science

Functional Characterization of the Antibiotic  
Resistance Reservoir in the Human Microflora

Morten O. A. Sommer<sup>\*,†</sup>, Gautam Dantas<sup>\*,†‡</sup>, George M. Church

# Functional metagenomics for resistome profiling

Is more frequently applied in metagenomic studies of environmental samples



## Discovery of Novel Antibiotic Resistance Determinants in Forest and Grassland Soil Metagenomes

Inka Marie Willms<sup>1</sup>, Aysha Kamran<sup>2</sup>, Nils Frederik Aßmann<sup>1</sup>, Denis Krone<sup>1</sup>, Simon Henning Bolz<sup>1</sup>, Fabian Fiedler<sup>1</sup> and Heiko Nacke<sup>1\*</sup>



Environment International  
Volume 132, November 2019, 105120



Novel clinically relevant antibiotic resistance genes associated with sewage sludge and industrial waste streams revealed by functional metagenomic screening

L. Zhang <sup>a, c, 1</sup>✉, L. Calvo-Bado <sup>a, d</sup>, A.K. Murray <sup>c</sup>, G.C.A. Amos <sup>a, e</sup>, P.M. Hawkey <sup>b</sup>, E.M. Wellington <sup>a</sup>, W.H. Gaze <sup>a, c, 1</sup>

## **Functional metagenomics for resistome profiling - limitations**

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The bacterial host must fit to the experimental setting, eg. not be intrinsically resistant to the antibiotic

Results fundamentally depend on the host's ability to express the cloned genes

The use of different media and incubation conditions make a direct comparison between studies difficult

Accurate quantification of ARGs is not possible

The choice of the insert size is an important factor, eg. resistance to antibiotics might be encoded by multiple genes

# Sequence-based metagenomics for resistome profiling

No need for prior amplification of a specific target gene

Unbiased characterization and quantification of the resistome

Metagenomic reads (or genes from assembled contigs) are mapped against a database with known ARGs

Confident matches against known ARGs can then be counted and quantified



## **Sequence-based metagenomics for resistome profiling**

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In metagenomic samples from the oral cavity ~2.8% of the total predicted genes coded for proteins involved in resistance to antibiotics and toxic compounds

The oral cavity may be an important reservoir for antimicrobial resistance

**molecular oral  
microbiology**



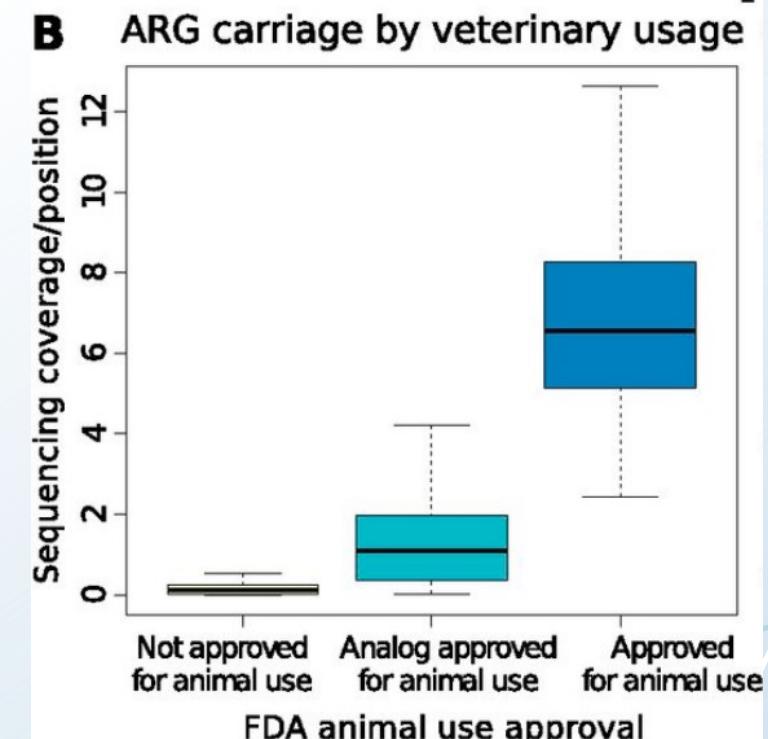
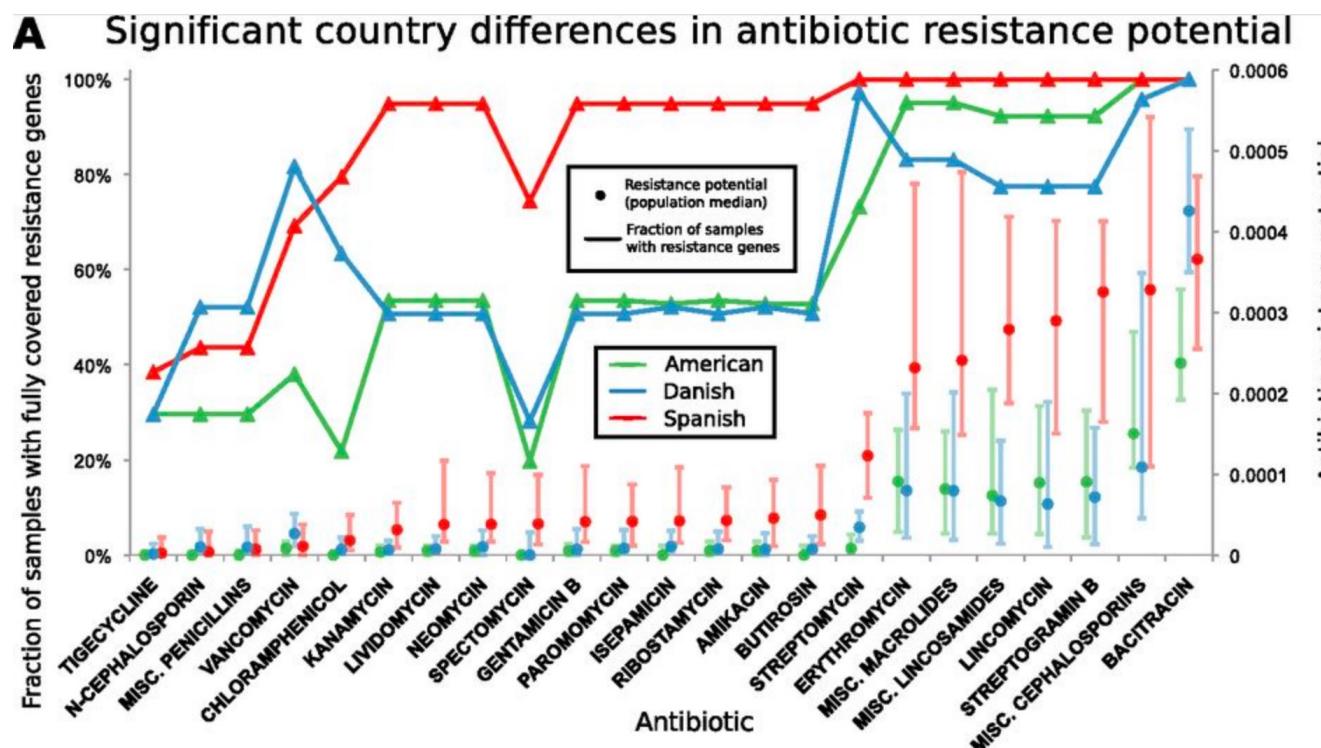
**Community and gene composition of a human dental plaque microbiota obtained by metagenomic sequencing**

G. Xie, P.S.G. Chain, C.-C. Lo, K.-L. Liu, J. Gans, J. Merritt, F. Qi

# Sequence-based metagenomics for resistome profiling

Analysis of gut microbiomes shows geographic differences in AGRs

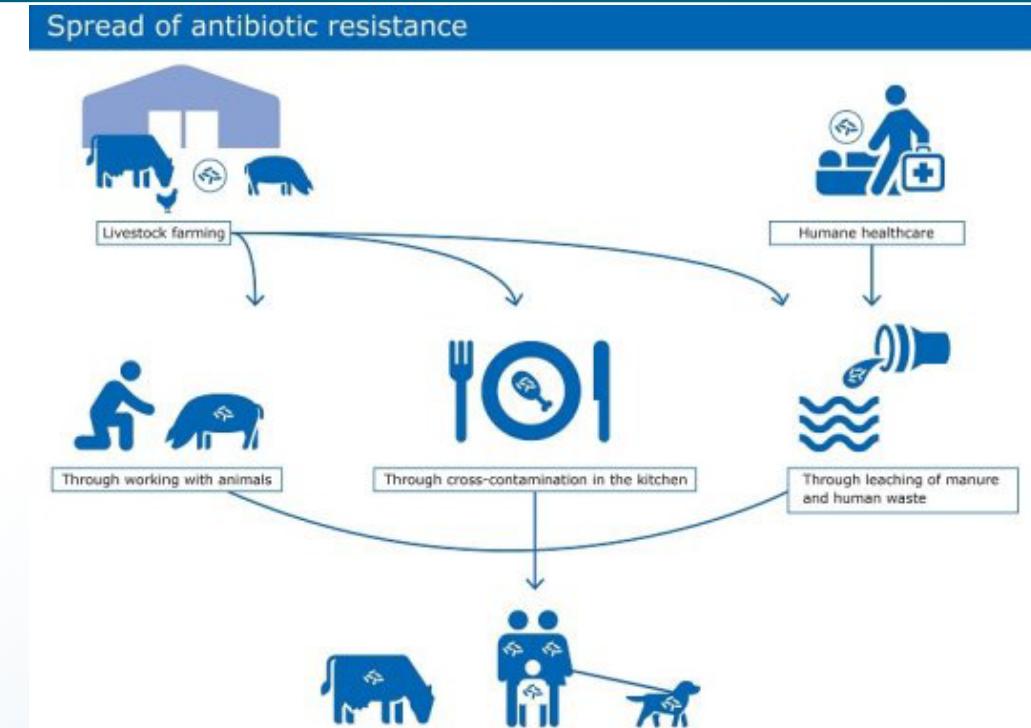
Administration of antibiotics in animals promotes the development of resistance in the human intestinal microbiota



# Sequence-based metagenomics allows tracking of the potential spread of AGRs

Sequence-based metagenomics for resistome profiling allow us to study the spread of AGRs

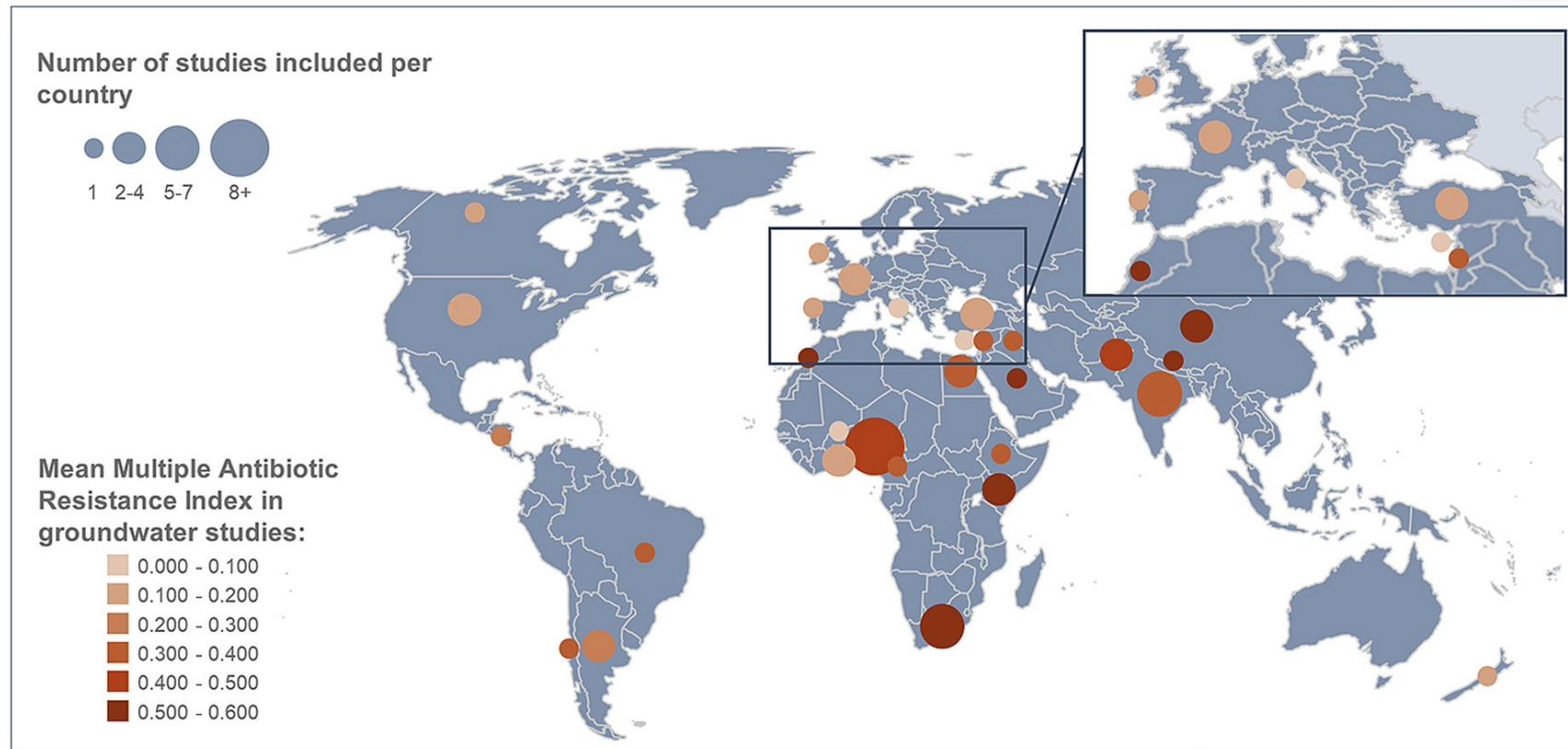
The use of antibiotics in farm animals enhances the selection for MDR bacteria, which increases the risk of transmission to humans.



Wageningen University & Research

# Sequence-based metagenomics allows potential AGR source and reservoir surveillance

Global analysis of antimicrobial-resistant bacteria in groundwater sources  
~30% of studied sources harboured resistant bacteria



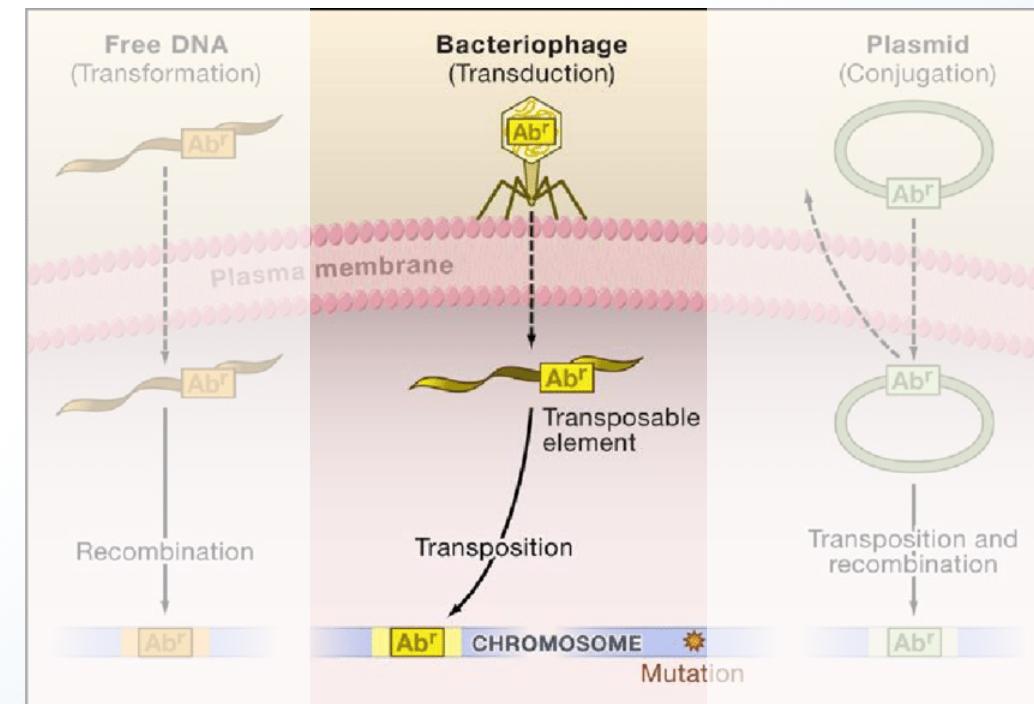
# Sequence-based metagenomics allows a broad range of phage-derived ARGs to be detected

Bacteriophages are often neglected in metagenomic analysis

Bacteriophages can be encountered everywhere in the environment and are highly abundant in water

Bacteriophages can persist in the environment

Bacteriophages might frequently act as vehicles for resistance transfer



Tula and Iruolaje. Antibiotic Resistance: Challenges and Prospect for Therapy in Developing Countries. British Journal of Pharmaceutical Research 8(3): 1-16, 2015, Article no.BJPR.19061 ISSN: 2231-2919

# **Sequence-based metagenomics for resistome profiling**

## **- limitations**

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ARGs highly dissimilar to sequences in databases cannot be detected (high database dependency)

No functional confirmation that a gene would actually confer the predicted resistance, only a reflection of its potential

Investigation of the genomic environment of an ARG is less reliable since usually based on a metagenomic assembly that is still not a standardized procedure

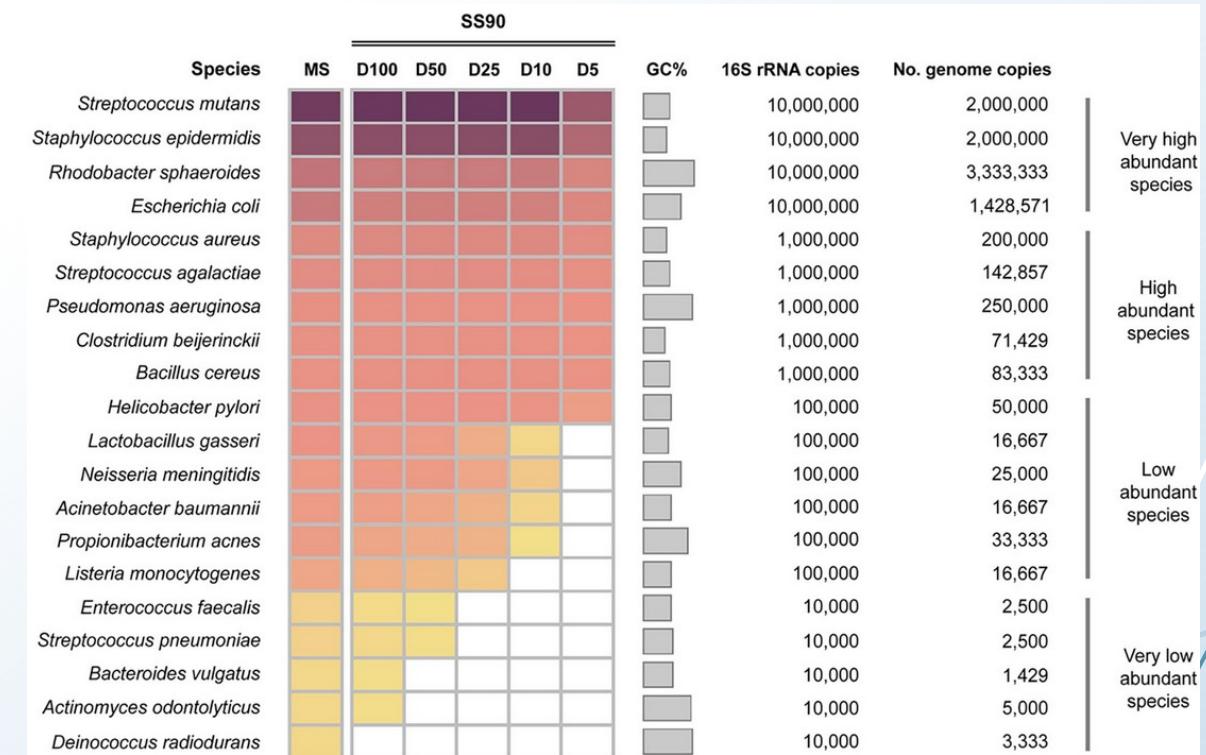
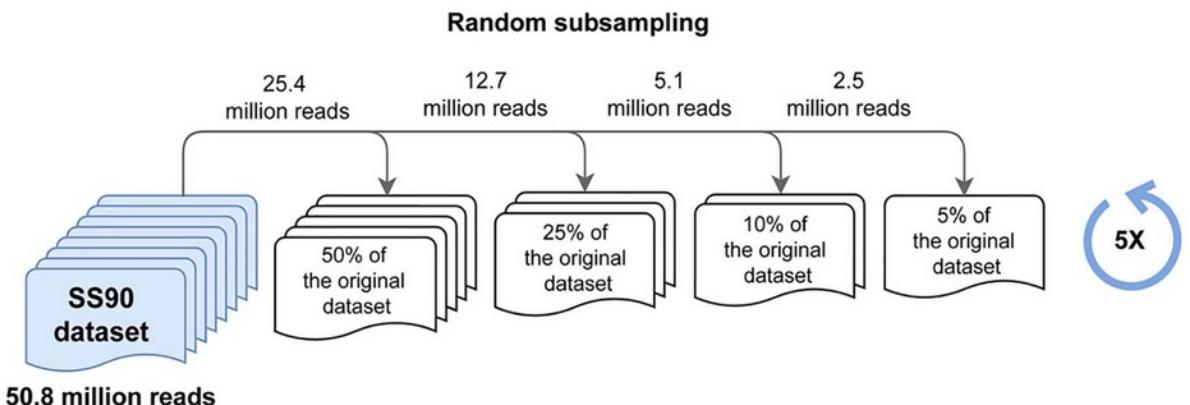
Short reads might accidentally align to ARGs when using less stringent mapping parameters

No standard criteria for pre-analytics, DNA extraction, sequencing and bioinformatic analysis available - Eg. optimum sequencing depth, data processing and analysis methods for samples

# Sequencing depth of metagenomic samples

The depth of sequencing has huge impact on the taxonomic resolution

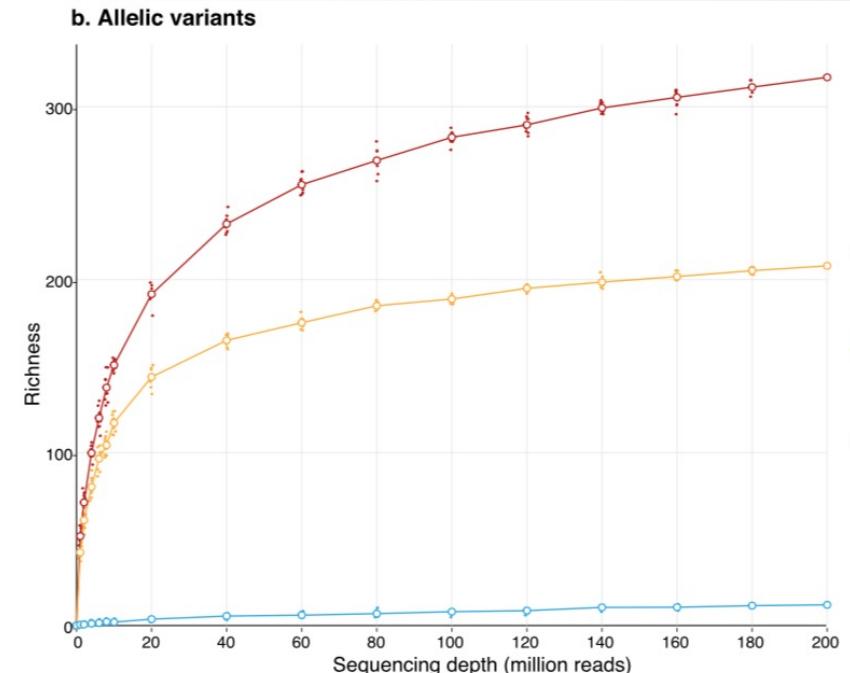
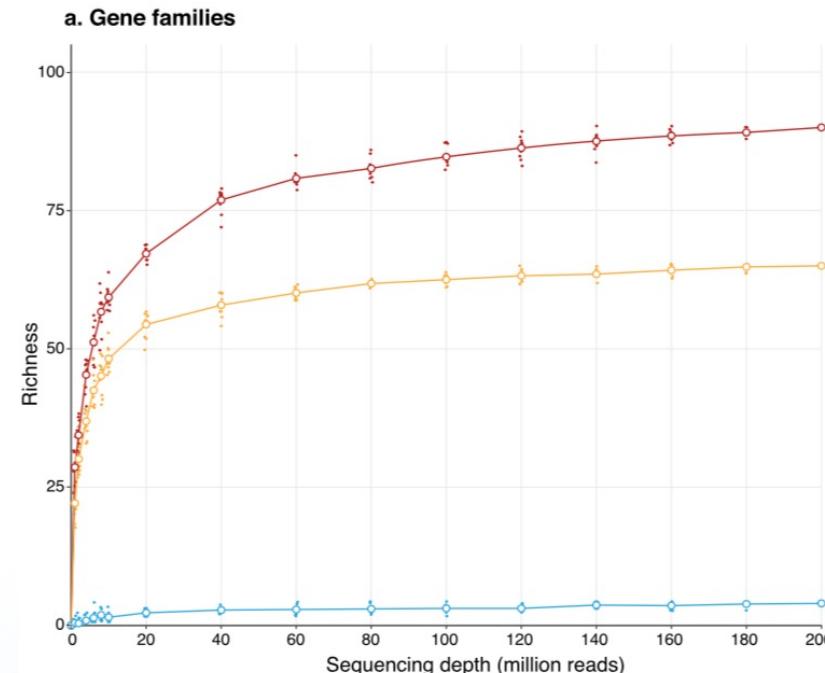
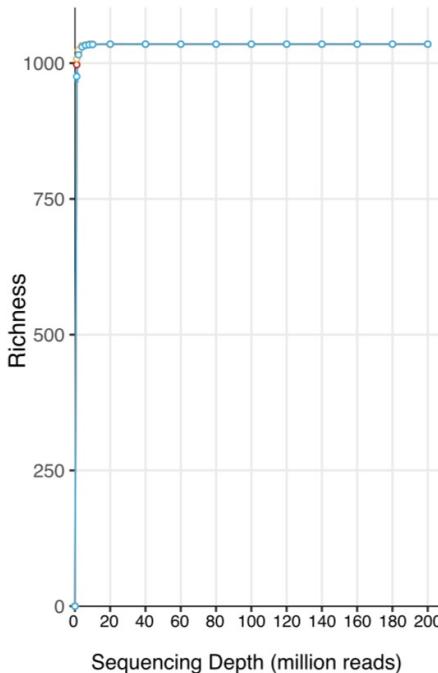
Taxonomic profiling becomes more inaccurate as the level of host DNA increases in a sample



# Sequencing depth of environmental metagenomic samples

Taxonomic profiling is much more stable to sequencing depth than AMR gene content

80 million reads per sample were required to recover the full richness of different AMR gene families



## RESEARCH ARTICLE

## Open Access

The impact of sequencing depth on the inferred taxonomic composition and AMR gene content of metagenomic samples

H. Soon Gweon<sup>1,2\*</sup>, Liam P. Shaw<sup>3</sup> , Jeremy Swain<sup>3</sup>, Nicola De Maio<sup>3</sup>, Manal AbuJoude<sup>4</sup>, Rene Niehus<sup>5</sup>, Alasdair T. M. Hubbard<sup>3</sup>, Mike J. Bowes<sup>2</sup>, Mark J. Bailey<sup>2</sup>, Tim E. A. Peto<sup>3,6</sup>, Sarah J. Hoosdally<sup>3</sup>, A. Sarah Walker<sup>3,6</sup>, Robert P. Sebra<sup>7</sup>, Derrick W. Crook<sup>3,8</sup>, Muna F. Anjum<sup>9</sup>, Daniel S. Read<sup>2</sup>, Nicole Stoesser<sup>3</sup> and on behalf of the REHAB consortium

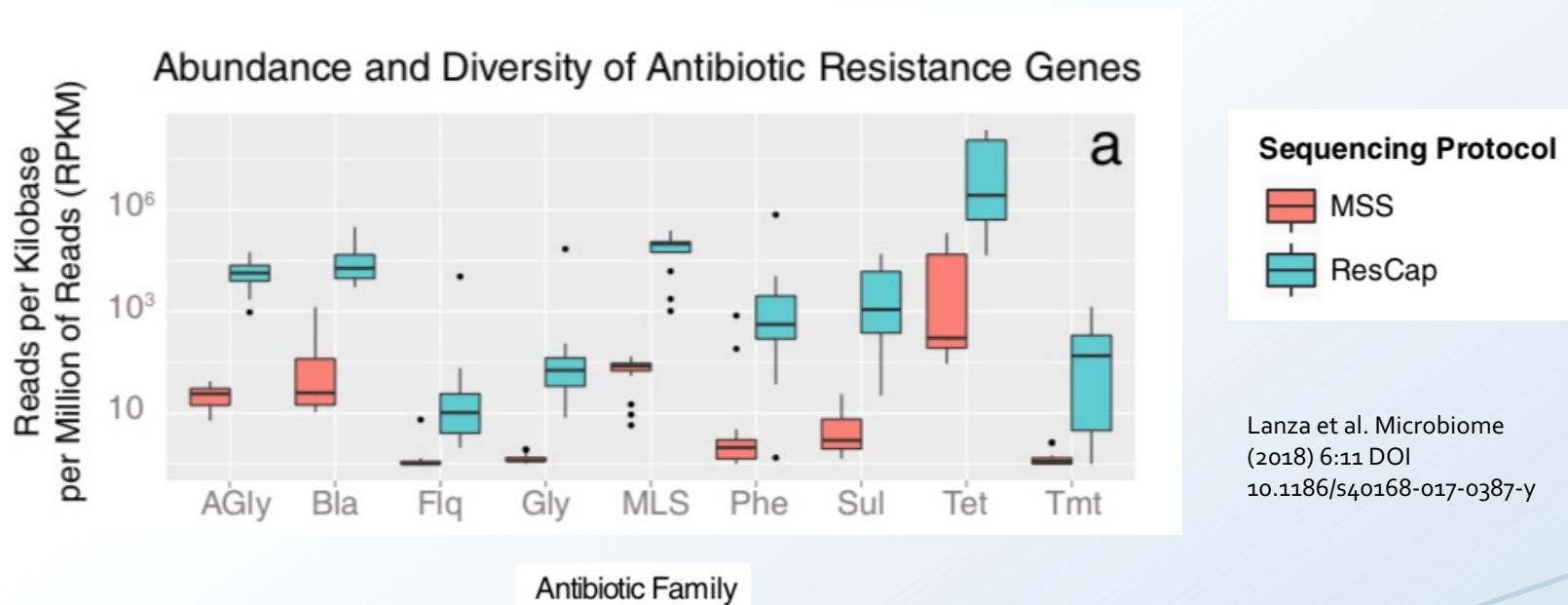


## Pros and cons

Functional based methods can identify novel ARGs

The resistome represents barely 0.1% of the gut metagenome

Sequence-based methods would need at least  $3.75 \times 10^9$  reads per sample to reach a similar coverage to that obtained by using Target capture methods (ResCap)



# Main ARG databases

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CARD, ARG-ANNOT, ARDB, RED-DB, ResFinder and Resfams

CARD uses a controlled vocabulary, the Antibiotic Resistance Ontology (ARO)



The screenshot shows the top navigation bar of the CARD website. It features a dark blue header with the word "CARD" in white. To the right are four links: "Browse", "Analyze", "Download", and "About". Below the header is a search bar with the placeholder "Search". On the left side of the header, there are two links in red: "Use or Download Copyright & Disclaimer" and "Help Us Curate #AMRCuration #WorkTogether".

## The Comprehensive Antibiotic Resistance Database

A bioinformatic database of resistance genes, their products and associated phenotypes.

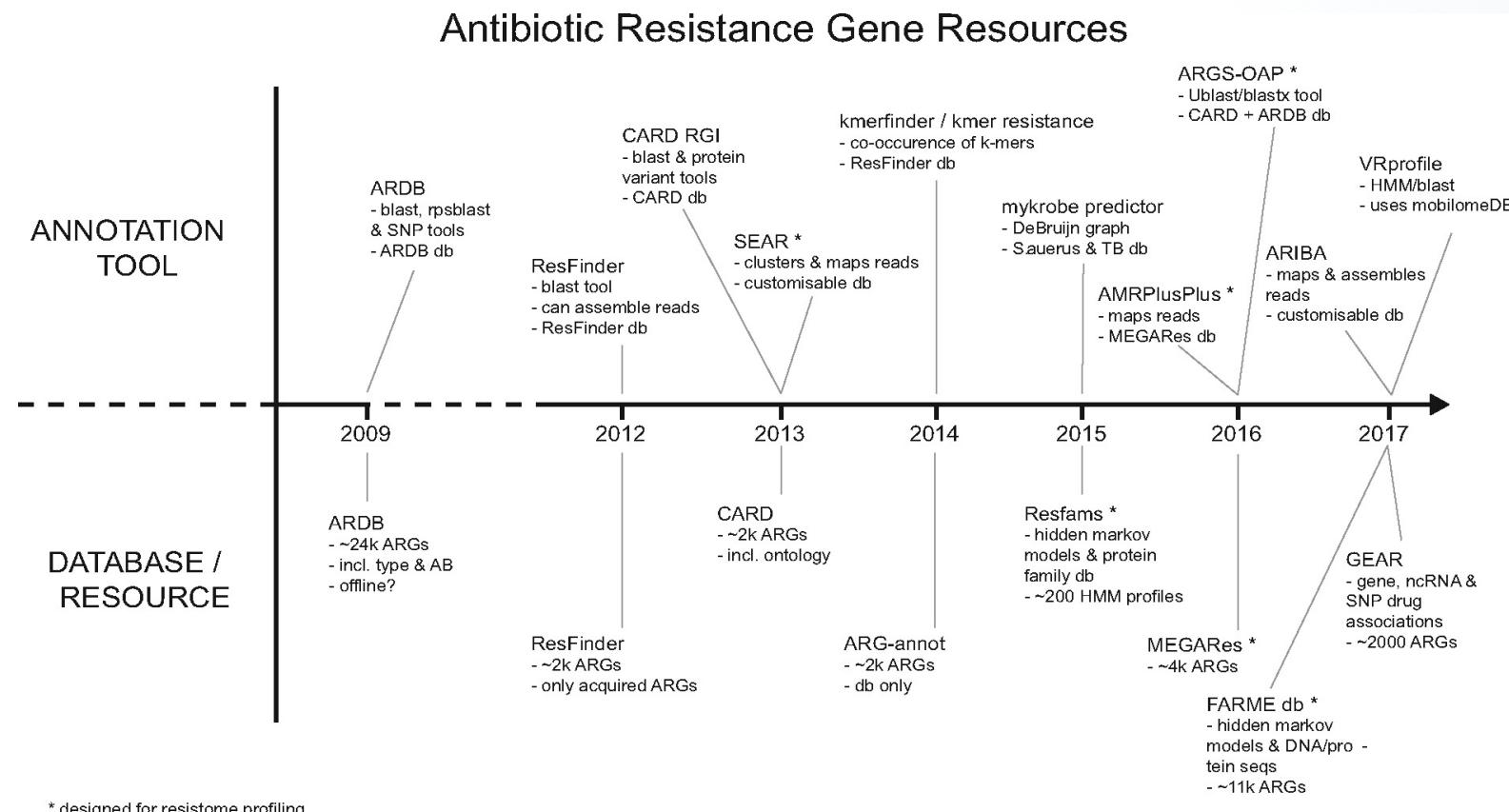
4358 Ontology Terms, 2909 Reference Sequences, 1318 SNPs, 2663 Publications, 2943 AMR Detection Models

Resistome predictions: 85 pathogens, 8046 chromosomes, 18337 plasmids, 90531 WGS assemblies, 182532 alleles

CARD is Updated Monthly | CARD Bait Capture Platform [Released](#)

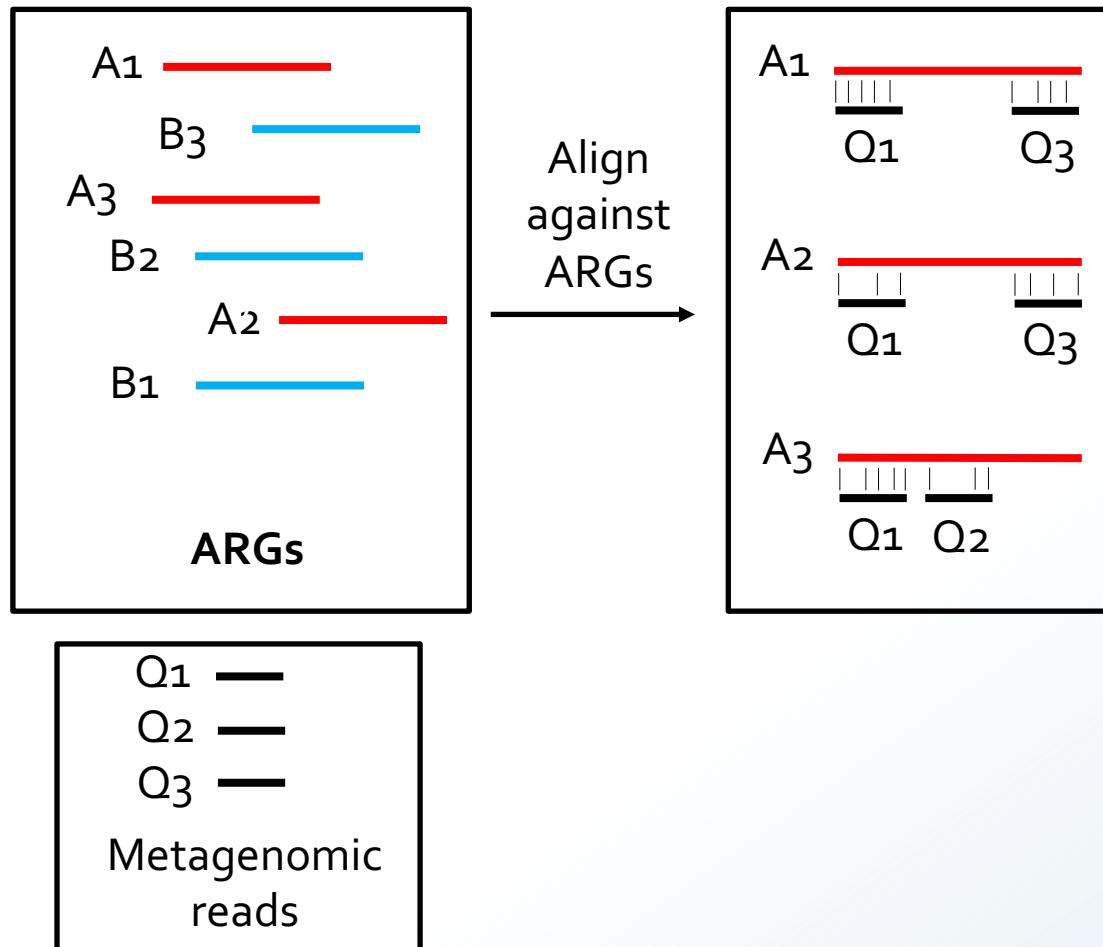
# Tools to identify ARGs in metagenomic samples by searching ARG databases

Tools and databases that are used to detect ARGs in sequencing data or assembled contigs



# Querying an AGR database - Homology search (Simplest method)

High similarity shared between reference sequences



**Disadvantages**

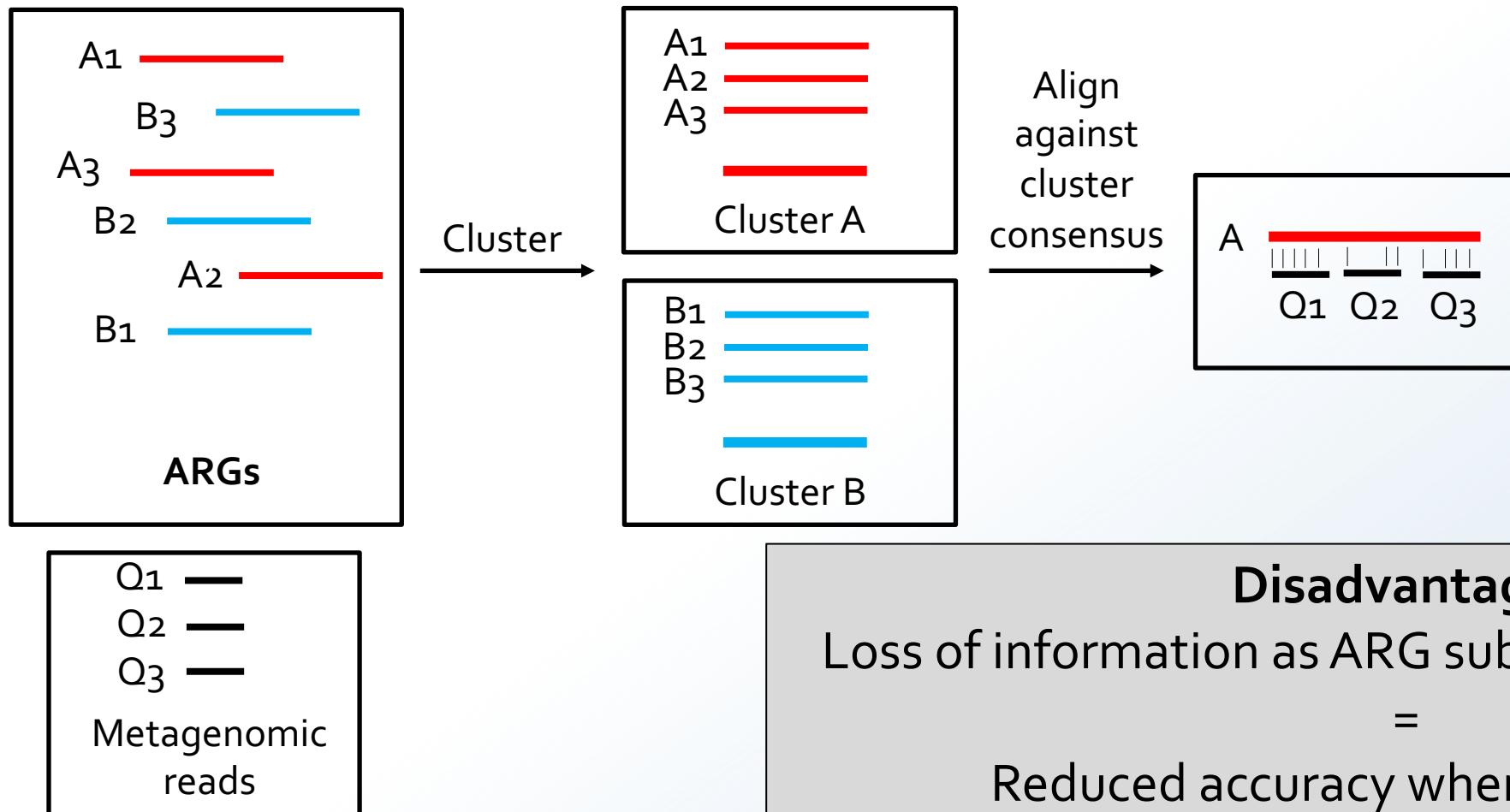
- Ambiguous alignments
- Unaligned reads (false negatives)
- Mis-annotated reads (false positives)

=

Reduced accuracy when typing ARGs

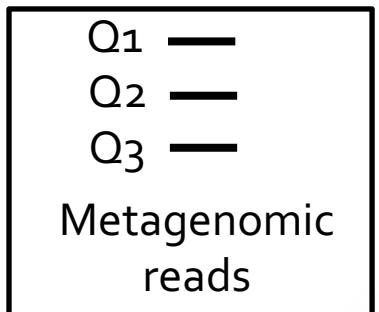
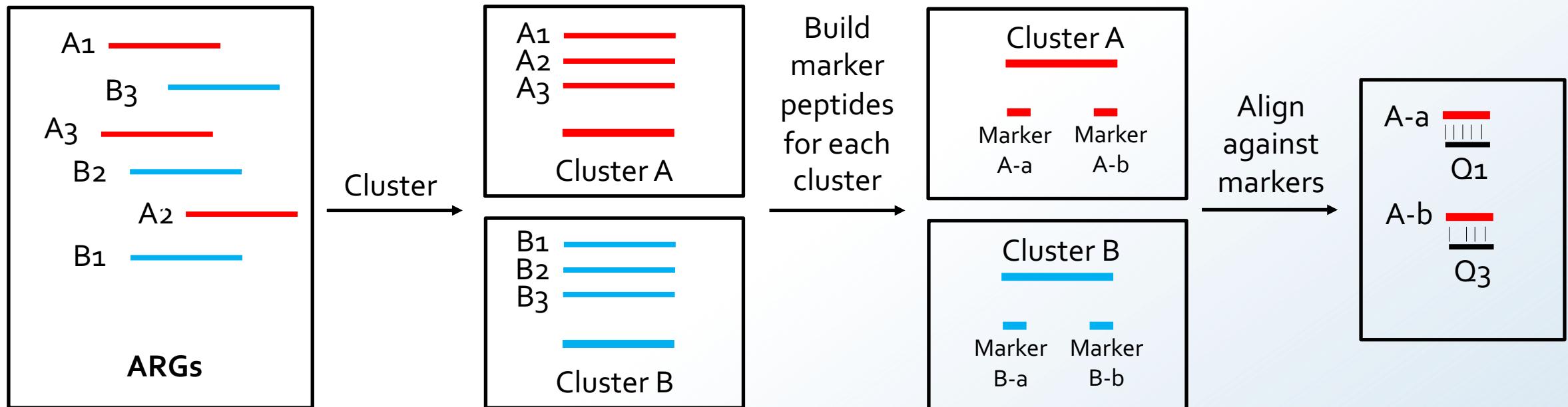
# Querying an AGR database - Cluster similar AGRs

The cluster consensus sequences are targets for read alignments



# Querying an AGR database - Cluster similar AGRs and construct marker peptides

Markers represent a family of ARGs



## Disadvantages

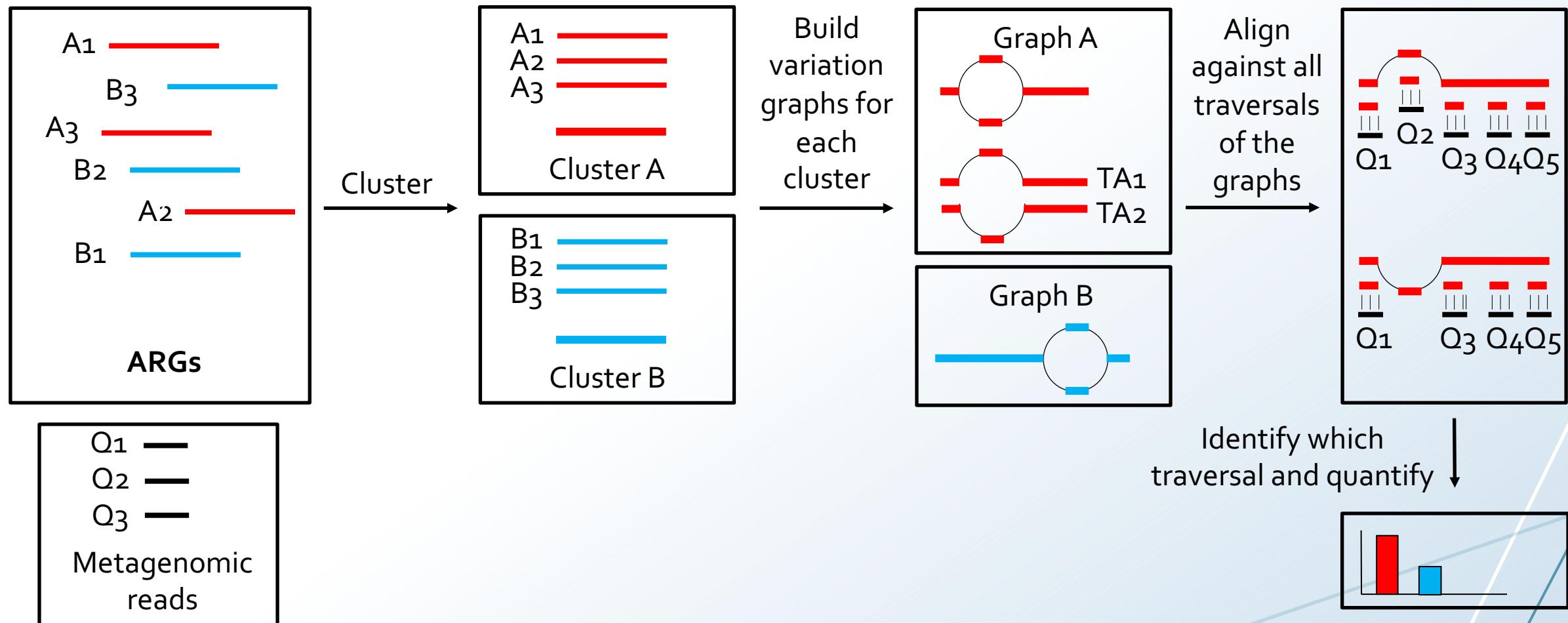
Loss of information as ARG subtypes will be masked

=

Reduced accuracy when typing ARGs

# Querying an AGR database - Cluster similar AGRs and construct variation graphs

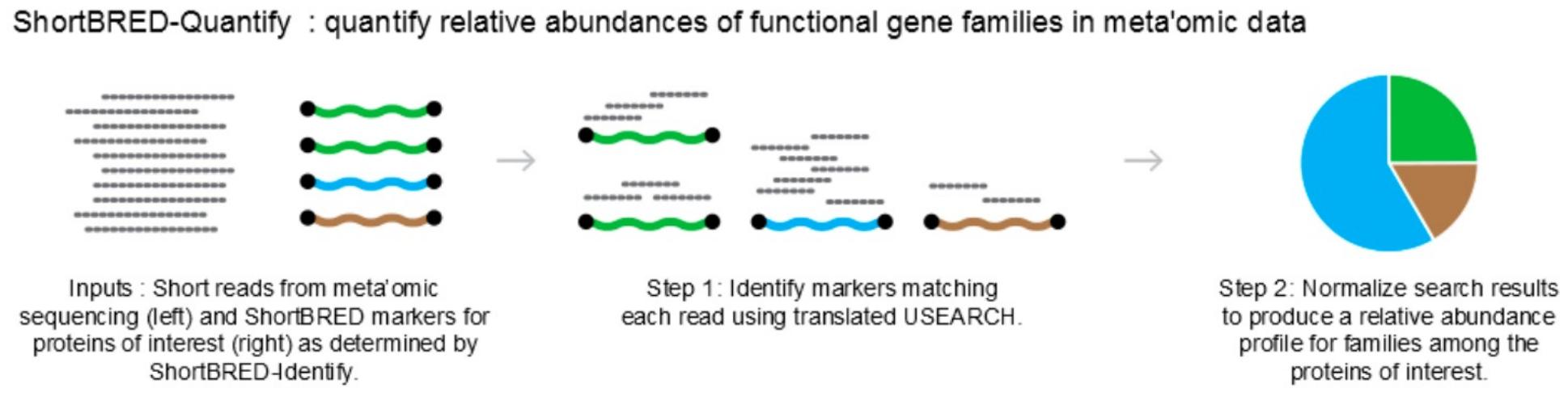
Variation graph represent an ARG cluster and retain sequence variations within a gene cluster



# ShortBRED: Short, Better Representative Extract Dataset

ShortBRED is a system for profiling protein families or AGRs in metagenomes

ShortBRED screens a metagenome against a given marker set to profile the presence/absence and relative abundance of the associated proteins

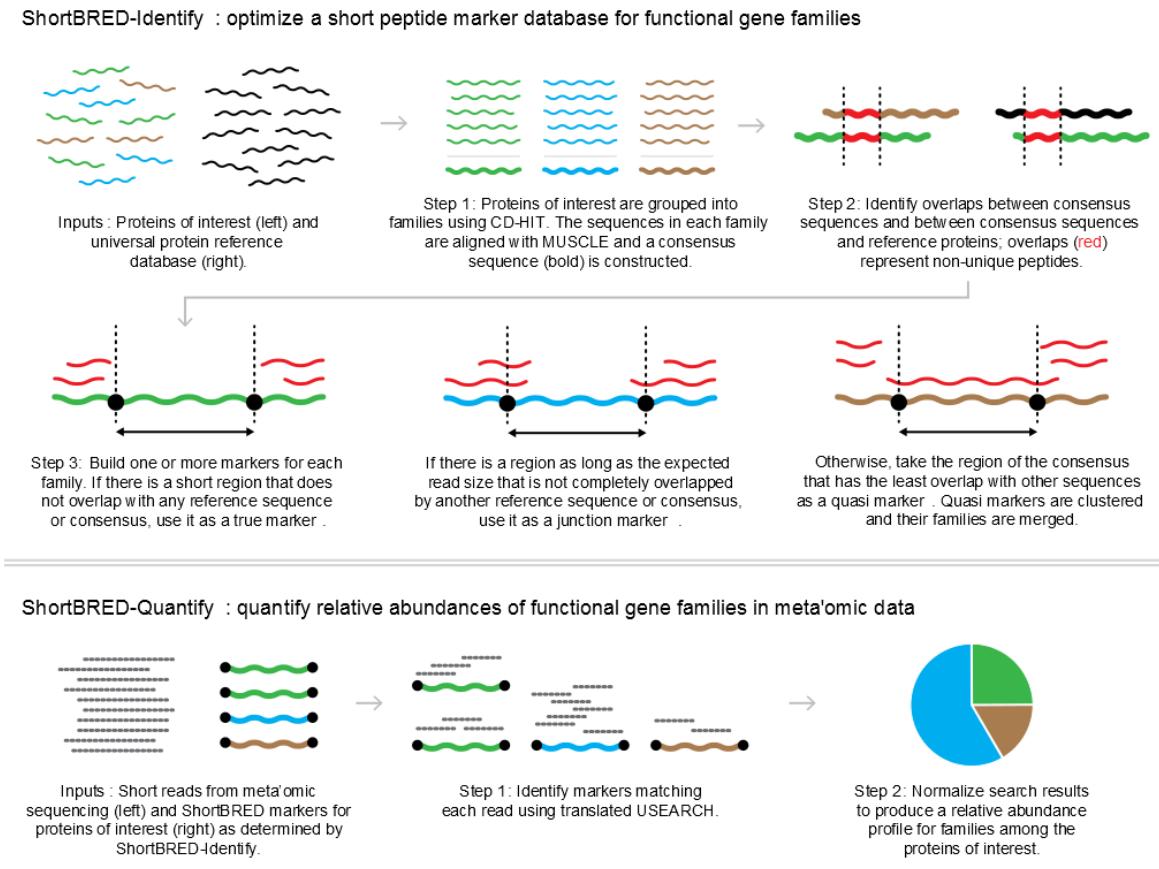


# ShortBRED: Short, Better Representative Extract Dataset

ShortBRED consists of two components:

1. A method that reduces reference proteins of interest to short, highly representative amino acid sequences ("markers")
2. A search step that maps reads to these markers to quantify the relative abundance of their associated proteins.

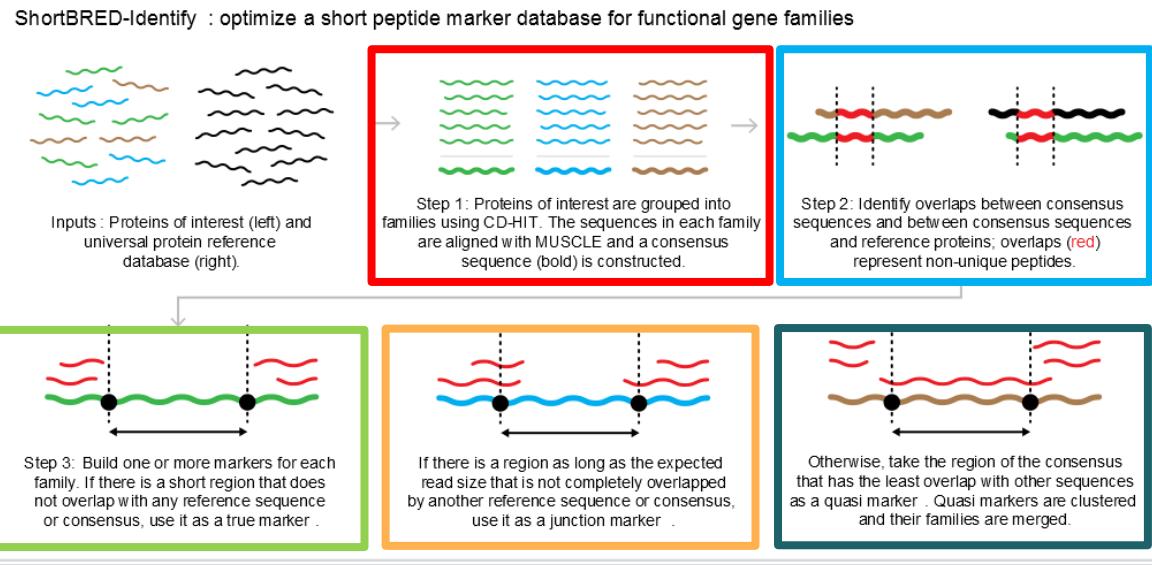
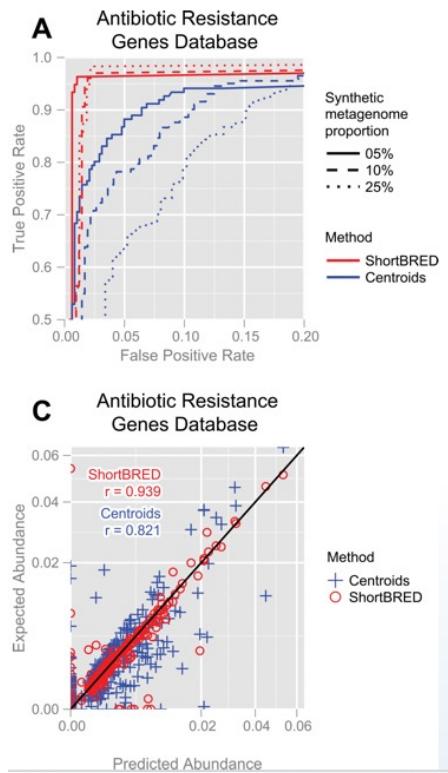
ShortBRED comes with pre-indexed databases



# ShortBRED: Short, Better Representative Extract Dataset

Generating markers for the ARDB

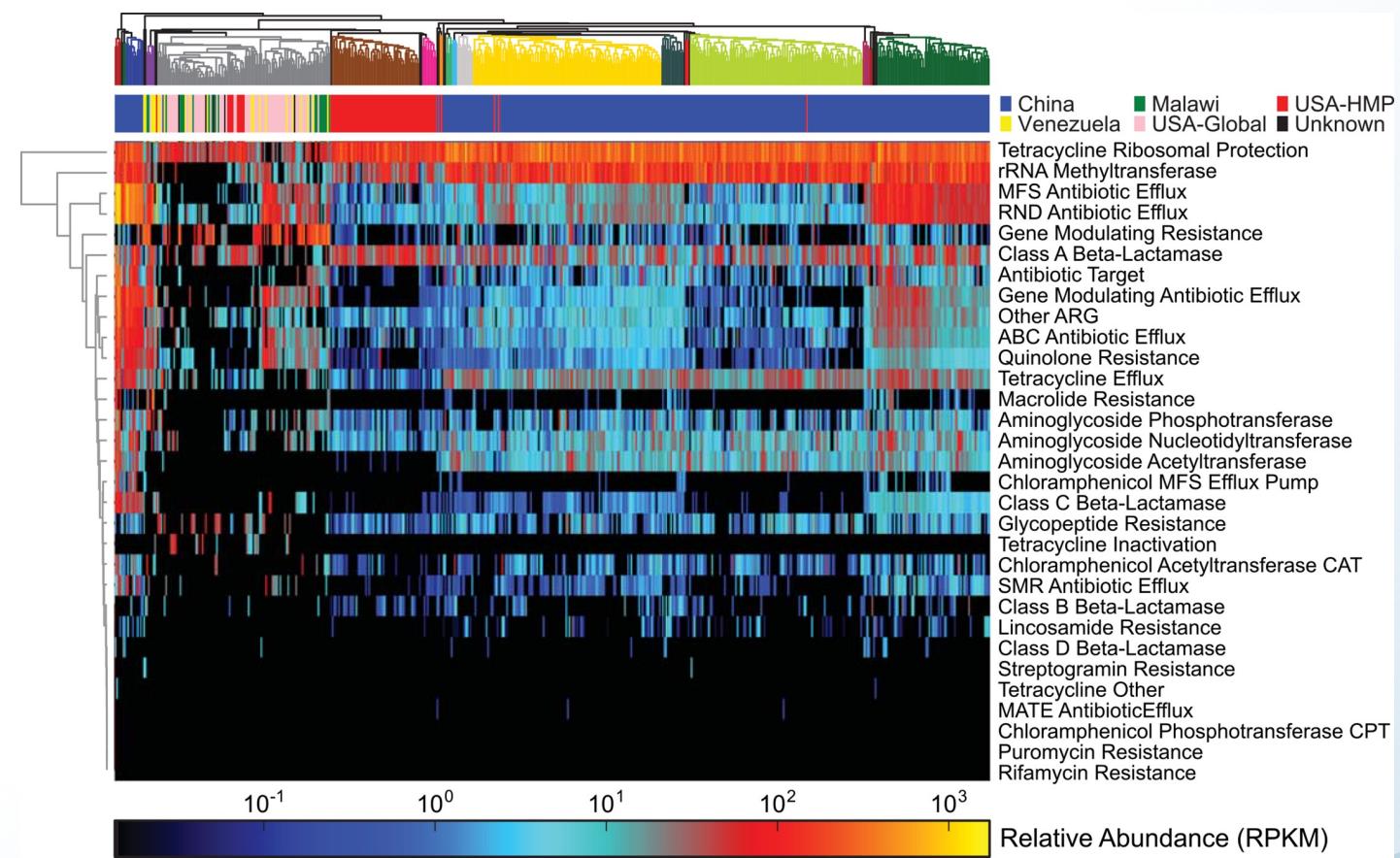
High sensitivity with very low false positives (<5%) – mock data



ARDB	
Families after initial clustering	618
Families with true markers	594
Families without true markers	24
Total markers	2,886
True markers	2,845
Junction markers	37
Quasi markers	4

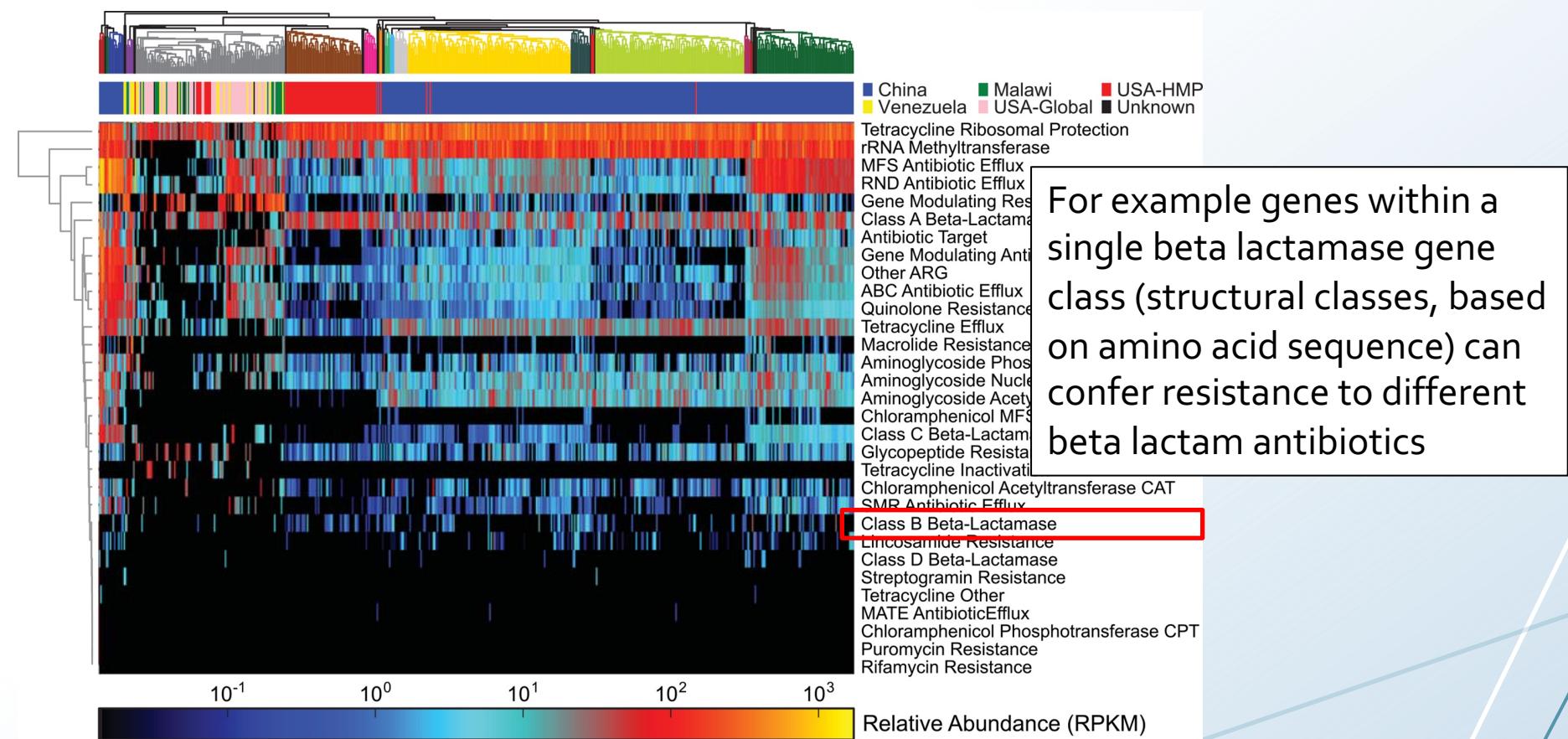
# ShortBRED: Short, Better Representative Extract Dataset

Applied to identify phylogenetic signatures of antibiotic resistance across more than 3,000 microbial isolate genomes



# Identification of ARG subtypes in a sample

Different ARG subtypes can provide selective resistance to different antibiotics



## **GROOT - graphing Resistance Out Of meTagenomes**

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Resistome profiling that utilizes a variation graph representation of ARG databases to reduce ambiguous alignment of metagenomic sequence reads

Sets of similar ARG reference sequences are stored in variation graphs; collapsing identical sequences whilst retaining unique nodes that allow for accurate typing

The non-linear reference representation reduces redundancy whilst maintaining information that facilitates classification

# GROOT - graphing Resistance Out Of meTagenomes

## For those who want to dig deeper into the method

