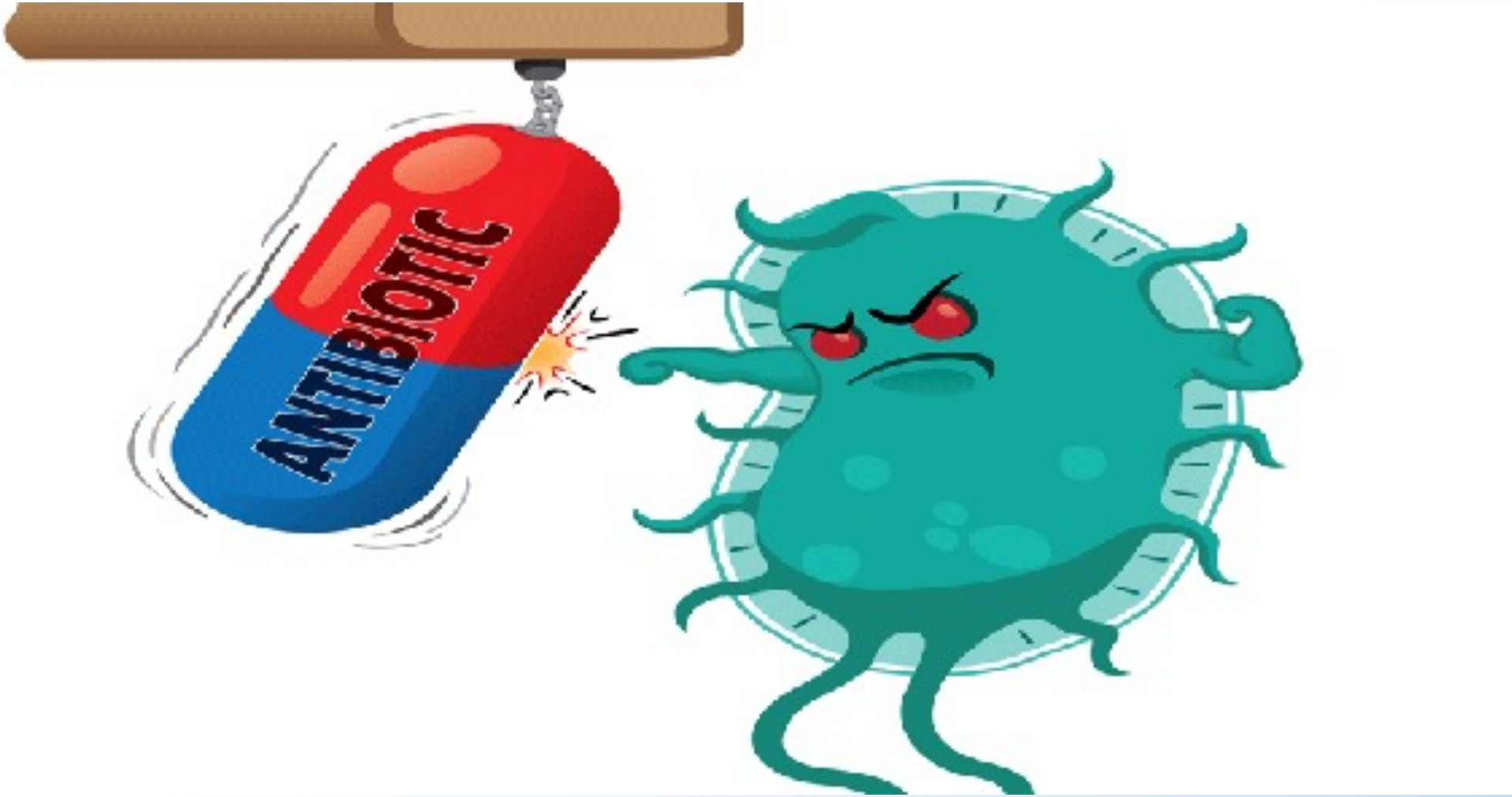
9. Resistome

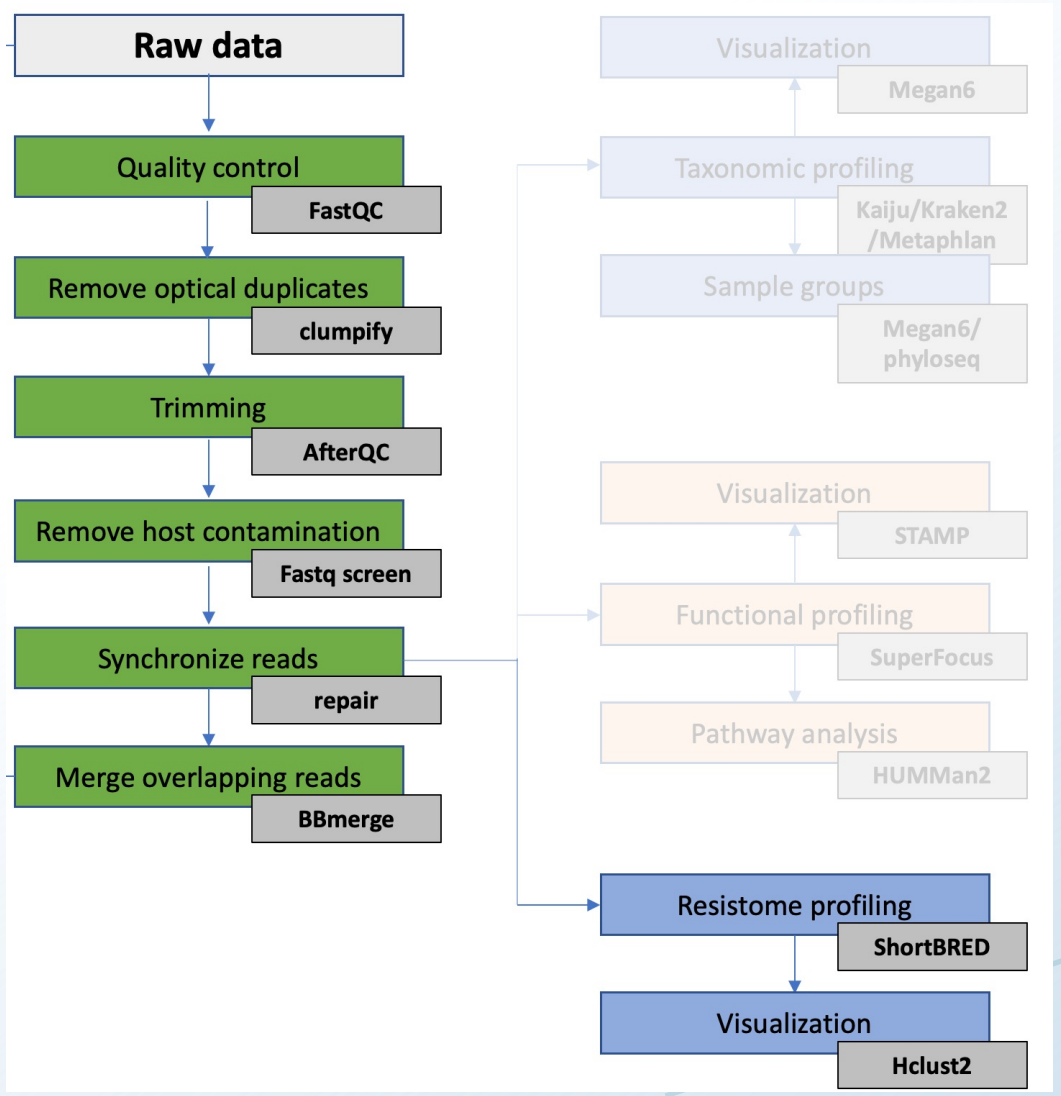


Overview of this talk

Antimicrobial resistance

Human resistome

Shortbred



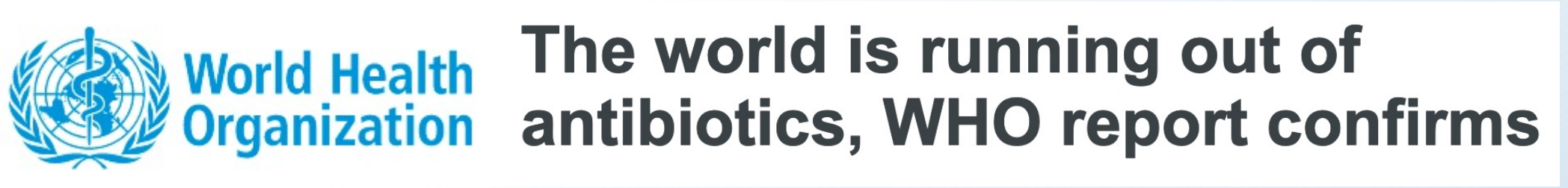
Antimicrobial resistance

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

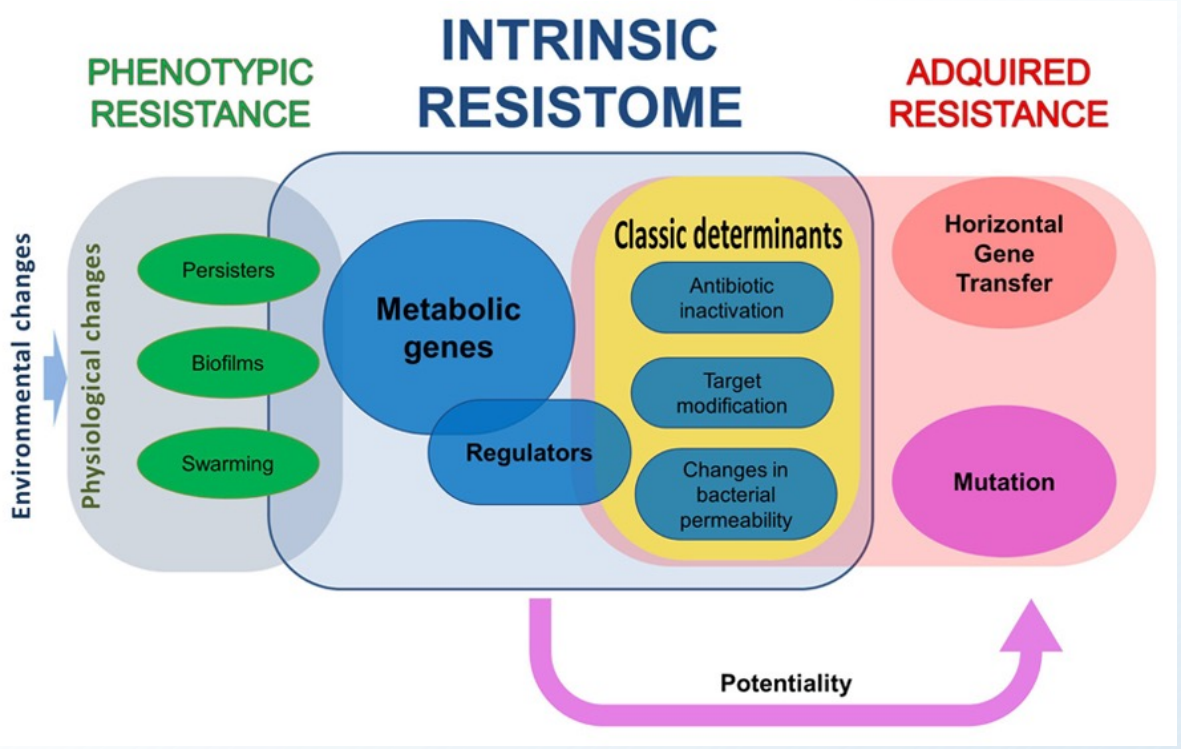


Acquired, intrinsic and phenotypic antimicrobial resistance

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



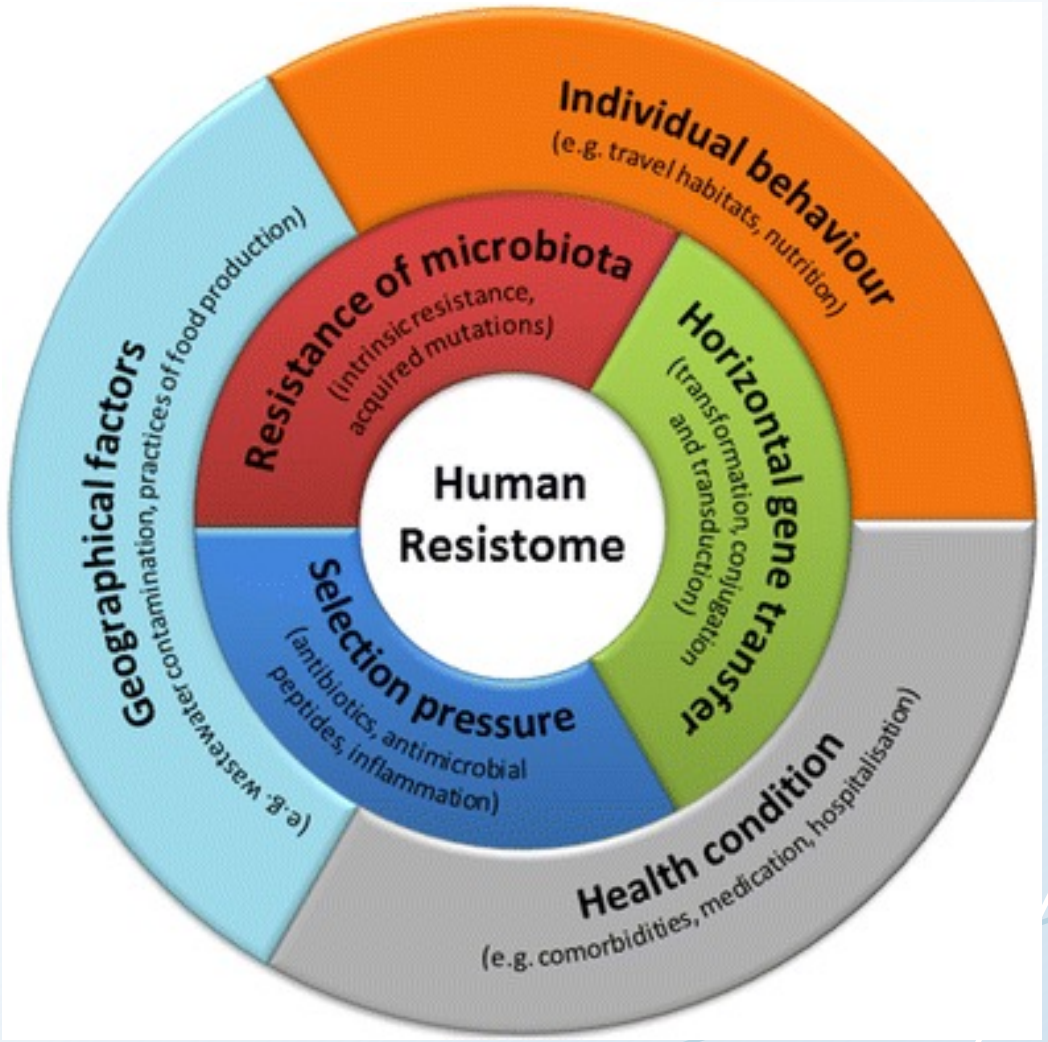
Olivares et al, The intrinsic resistome of bacterial pathogens. Front. Microbiol., 30 April 2013 | https://doi.org/10.3389/fmicb.2013.00103

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



Journal of Molecular Medicine. January 2017, Volume 95, Issue 1, pp 41–51

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

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

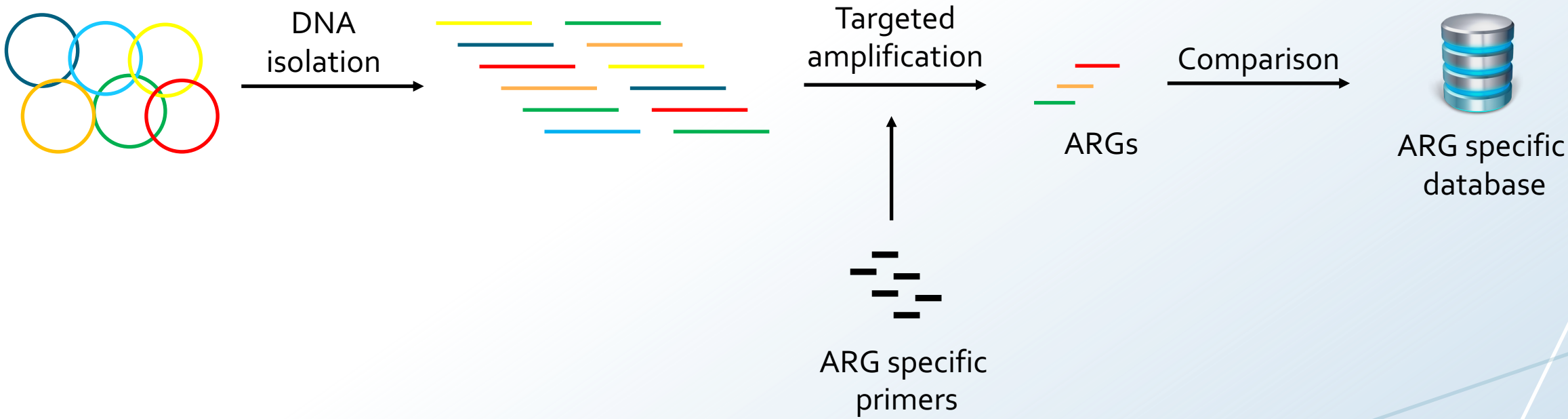
- Lack of bias
- Less sensitive than targeted, culture-based methods
- Allows for a 'One Health' approach - considering human, animal and environmental AMR reservoirs

Targeted (PCR-based) metagenomics for resistome profiling

PCR amplification of ARGs of interest

Limited to track known ARGs

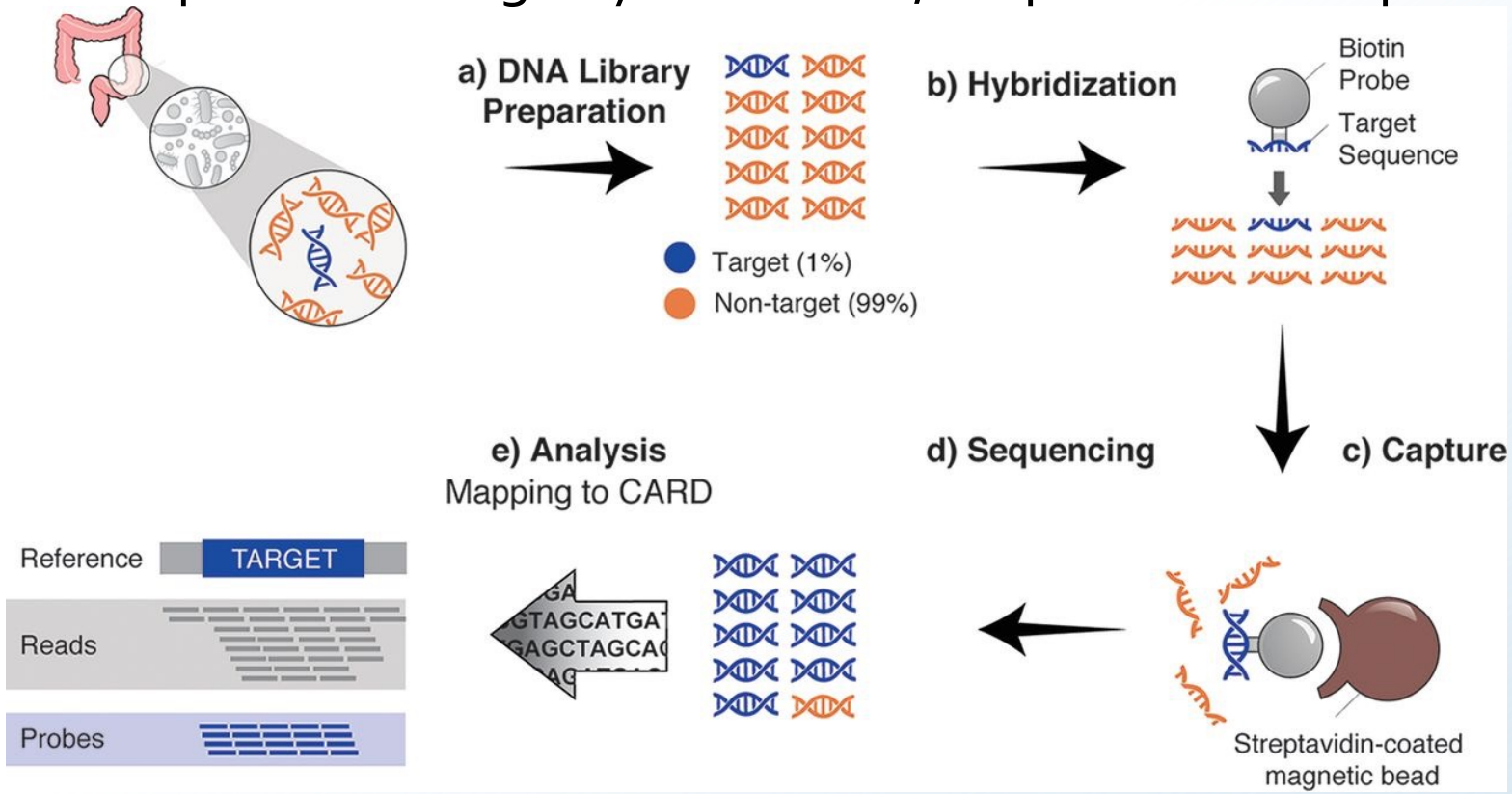
Do not give any information regarding the bacterial host of the ARGs



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



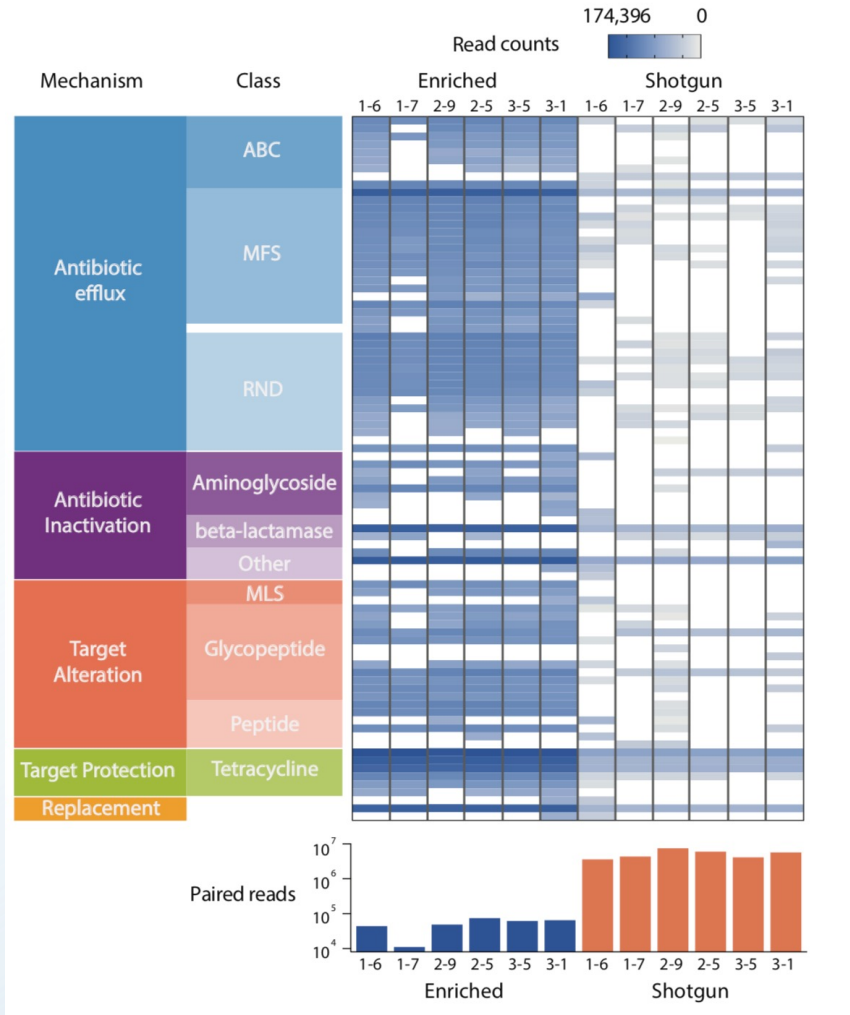
Guitor et al. Capturing the Resistome: a Targeted Capture Method To Reveal Antibiotic Resistance Determinants in Metagenomes. Antimicrob Agents Chemother. 2019 Dec 20;64(1). pii: e01324-19. doi: 10.1128/AAC.01324-19. Print 2019 Dec 20.

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

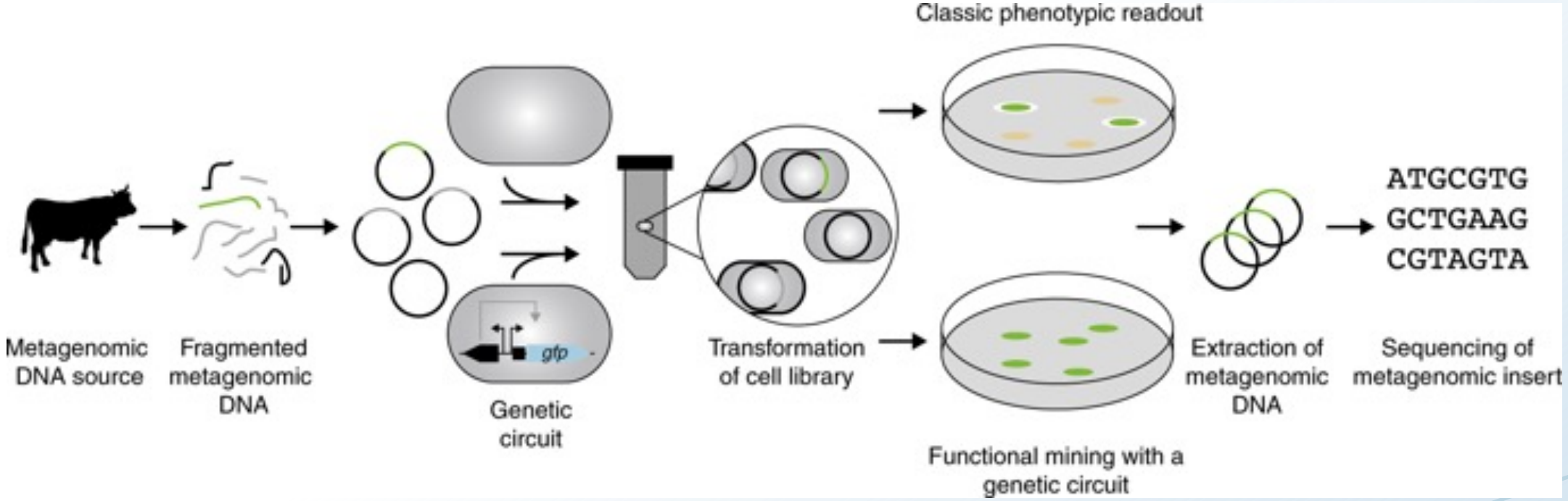
This method to capture the resistome enables a sensitive means of gene detection



Functional metagenomics for resistome profiling

Does not require any prior knowledge about the resistance genes

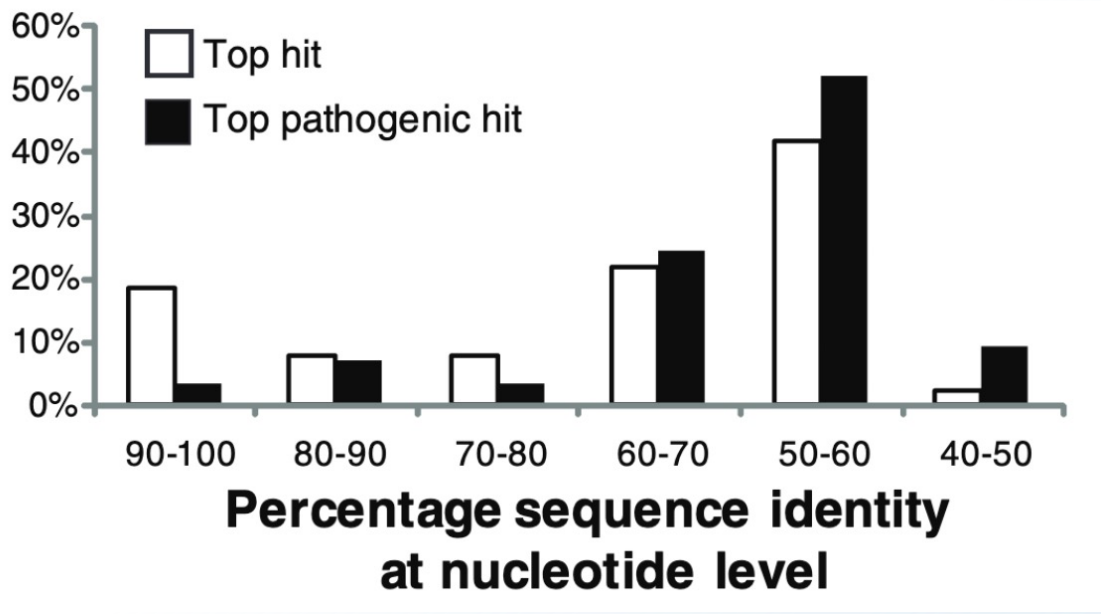
Only metagenomics method available which allows the isolation of completely novel AGRs on a large scale



van der Helm, E., Genee, H.J. & Sommer, M.O.A. The evolving interface between synthetic biology and functional metagenomics. *Nat Chem Biol* **14,** 752–759 (2018). https://doi.org/10.1038/s41589-018-0100-x

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*,†, Gautam Dantas*,†‡, 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[1], Aysha Kamran[2], Nils Frederik Aßmann[1], Denis Krone[1], Simon Henning Bolz[1], Fabian Fiedler[1] and Heiko Nacke[1*]

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 [a, c, 1], L. Calvo-Bado [a, d], A.K. Murray [c], G.C.A. Amos [a, e], P.M. Hawkey [b], E.M. Wellington [a], W.H. Gaze [a, c, 1]

Functional metagenomics for resistome profiling - limitations

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

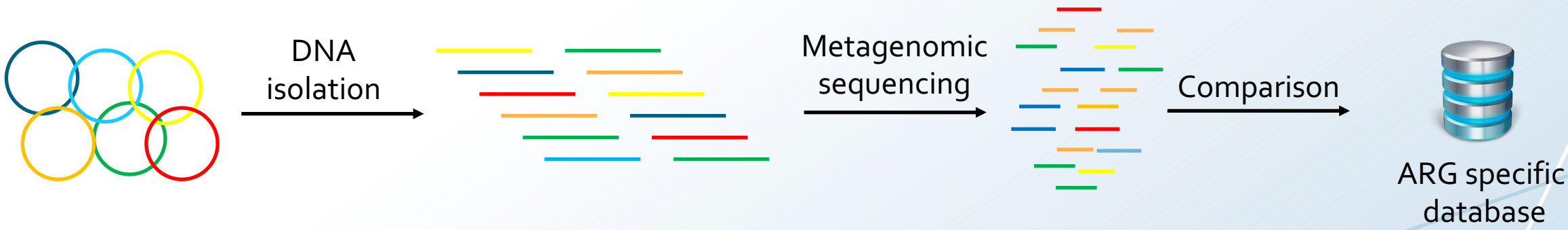
Sequence-based metagenomics for resistome profiling

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 AGRs

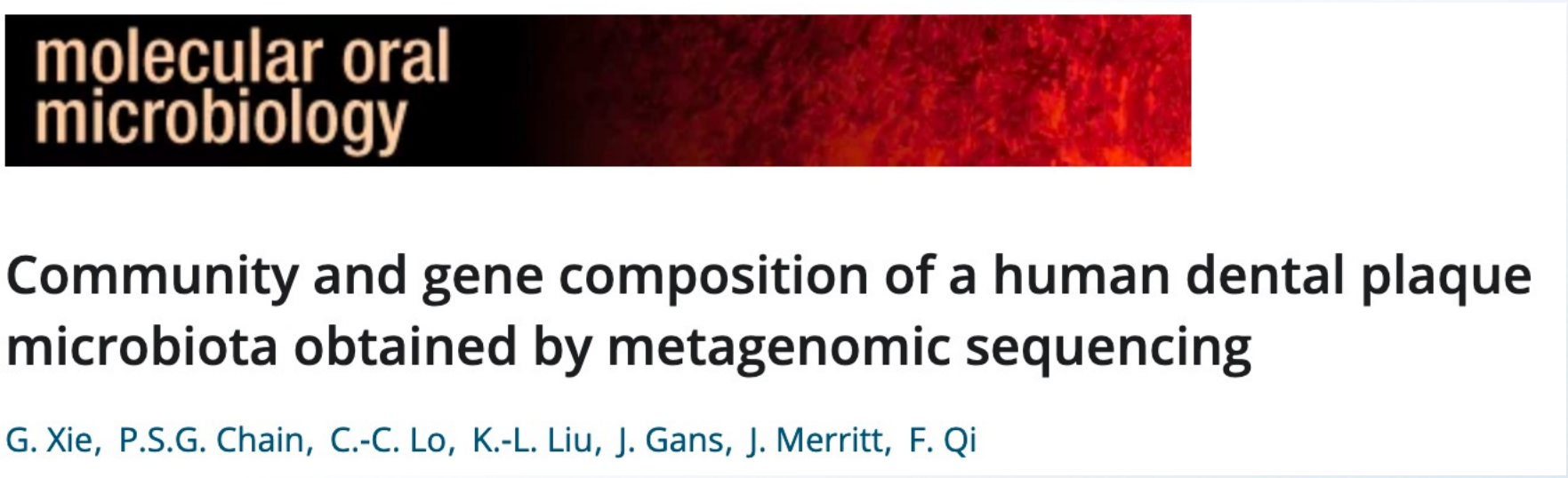
Confident matches against known ARGs can then be counted and quantified



Sequence-based metagenomics for resistome profiling

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

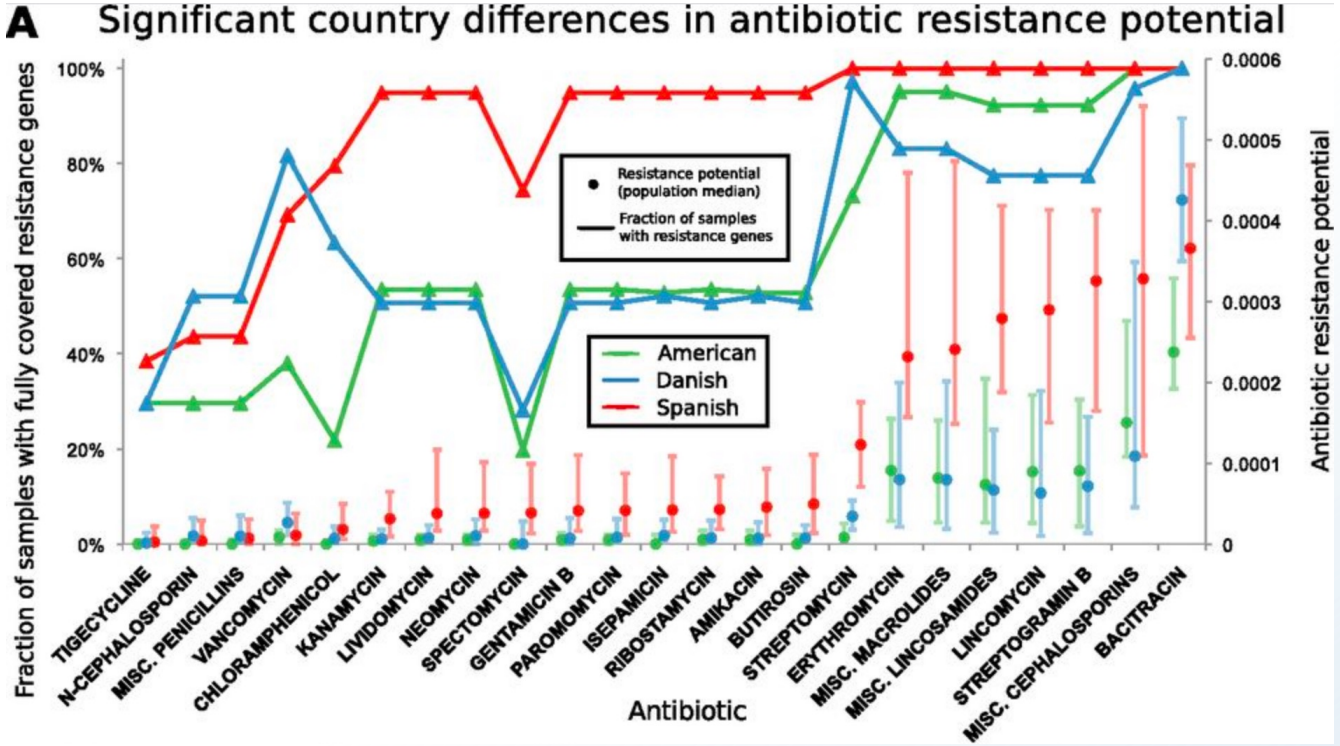
The oral cavity may be an important reservoir for antimicrobial resistance

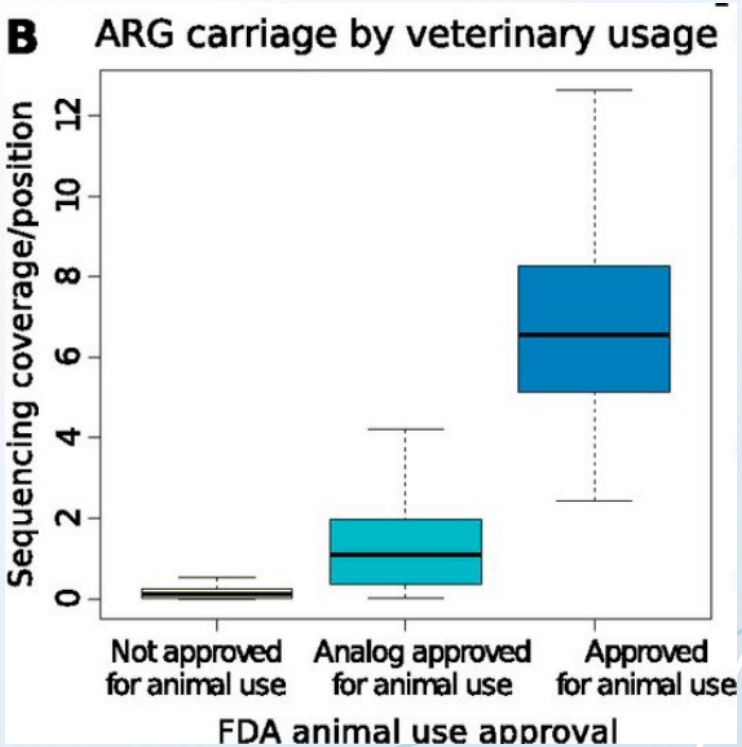


Sequence-based metagenomics for resistome profiling

Analysis of gut microbiomes shows geographic differences in AGRs

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



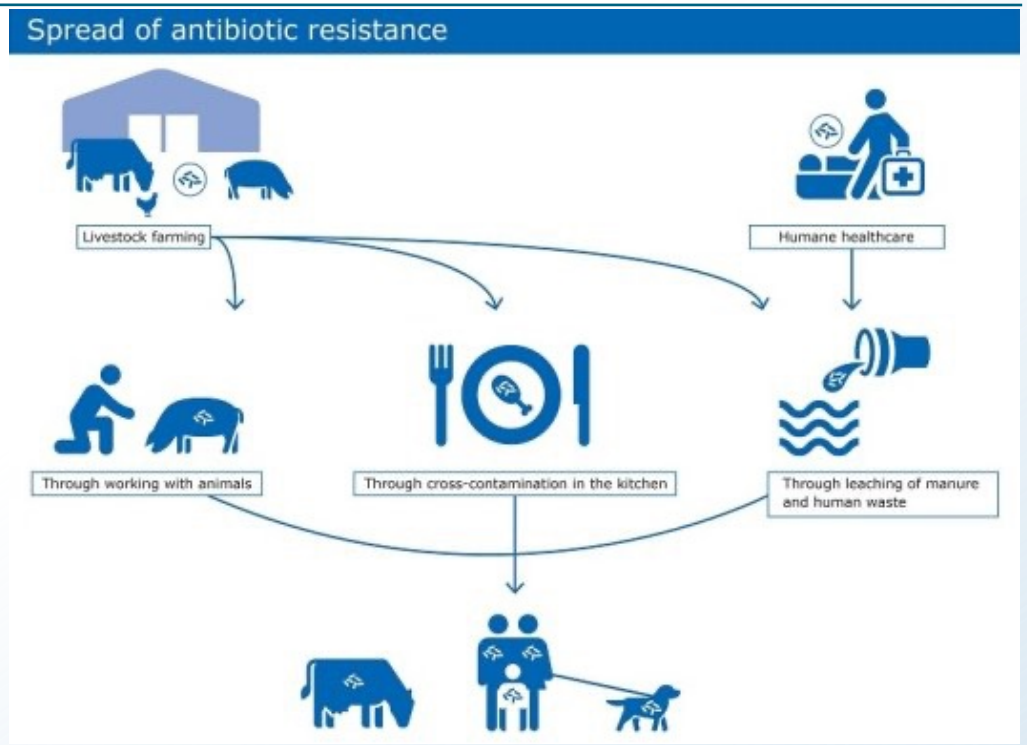


Forslund et al. Country-specific antibiotic use practices impact the human gut resistome. Genome Res. 2013. 23: 1163-1169. doi: 10.1101/gr.155465.113

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.

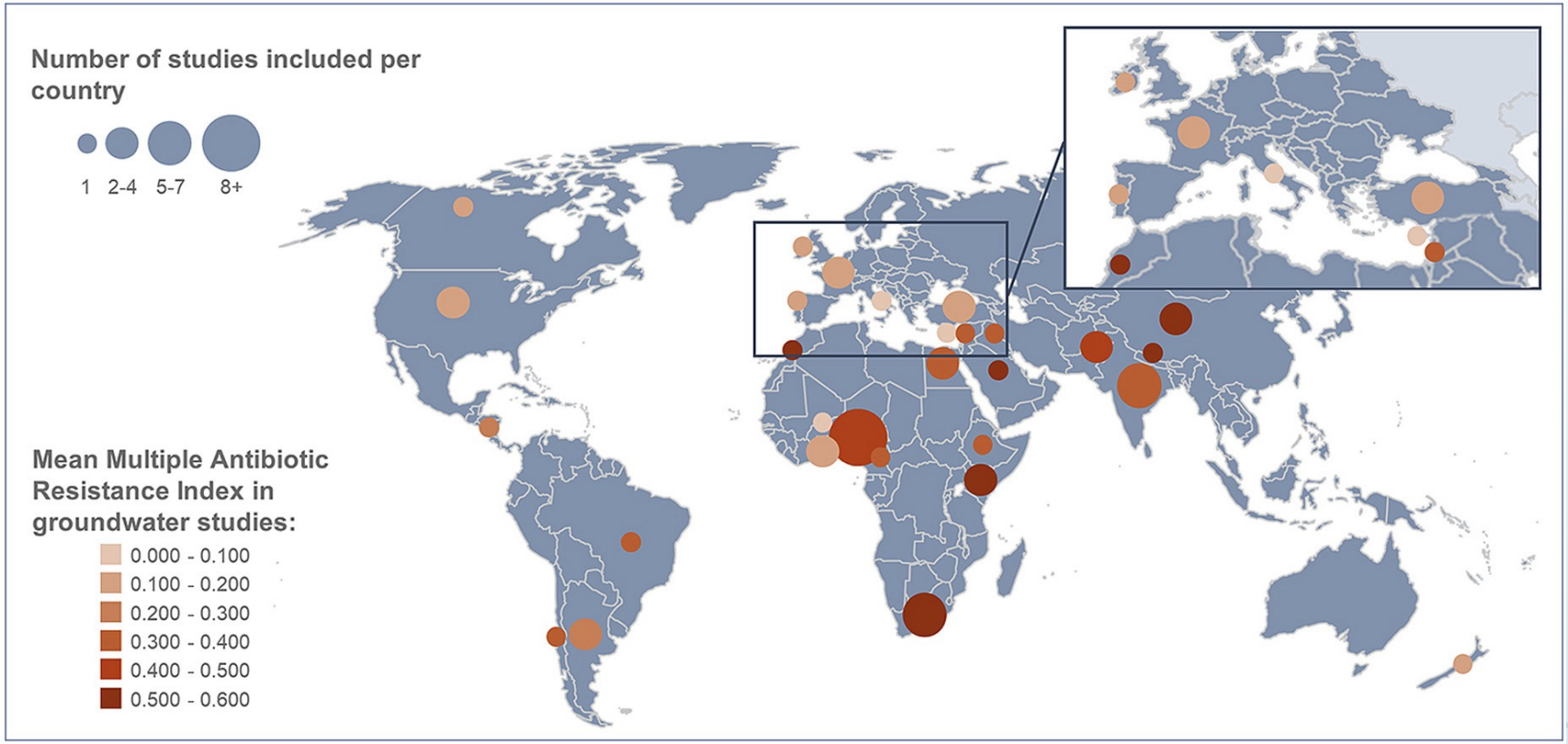


Wageningen University & Research

Sequence-based metagenomics allows potential AGR source and reservoir surveilance

Global analysis of antimicrobial-resistant bacteria in groundwater sources

~30% of studied sources harboured resistant bacteria



Andraade et al. Groundwater resources as a global reservoir for antimicrobial-resistant bacteria. Water Research. Volume 170, 1 March 2020, 115360. https://doi.org/10.1016/j.watres.2019.115360

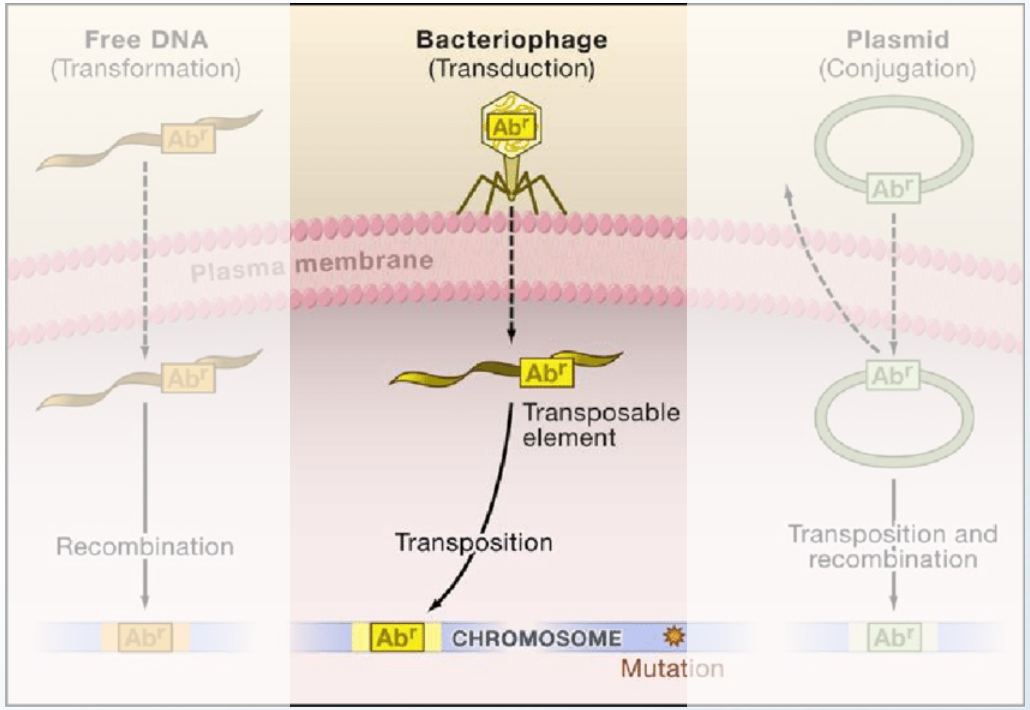
Sequence-based metagenomics allows a broad range of phage-derived ARGs to be detected

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

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

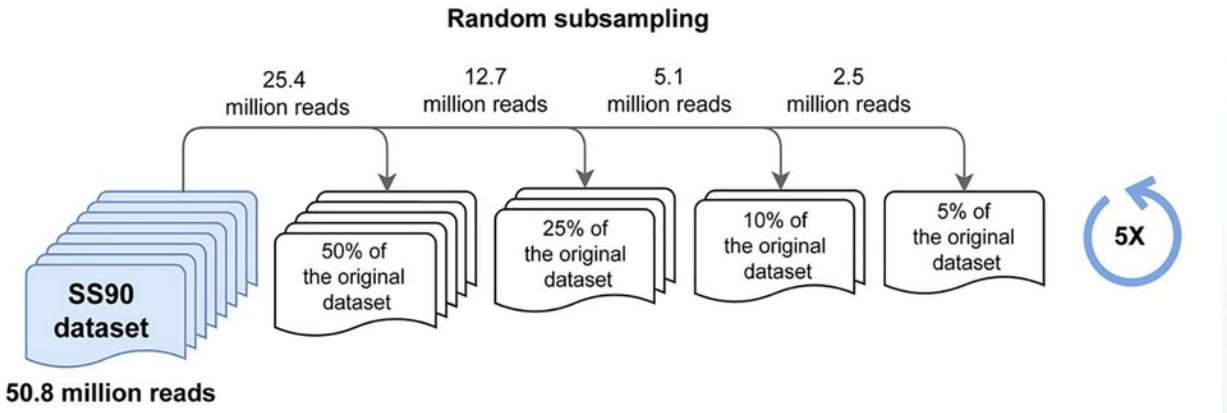
Short reads might accidently align to ARGs when using less stringent mapping parameters

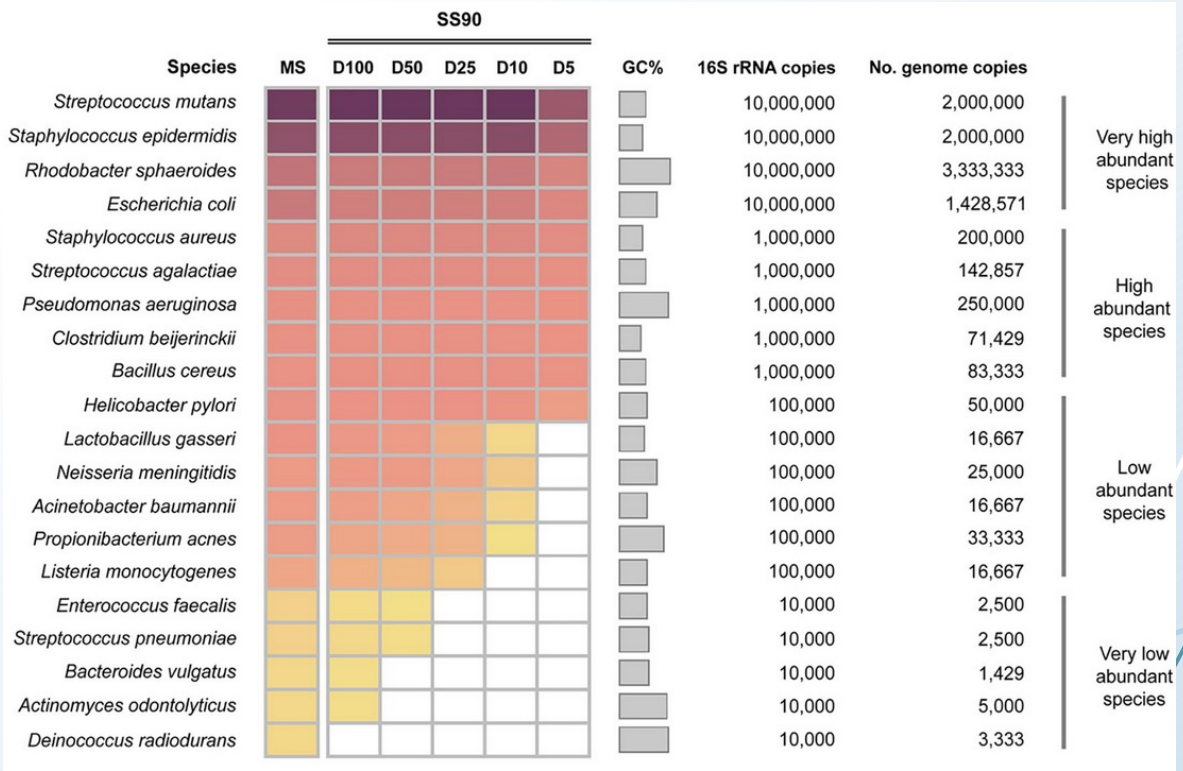
No standard criteria for pre-analytics, DNA extraction, sequencing and bioinformatic analysis available - Eg. optimum sequencing depth, data processing and analysis methods for samples

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





Pereira-Marques et al. Impact of Host DNA and Sequencing Depth on the Taxonomic Resolution of Whole Metagenome Sequencing for Microbiome Analysis. Front. Microbiol., 12 June 2019 | https://doi.org/10.3389/fmicb.2019.01277

Sequencing depth of environmental metagenomic samples

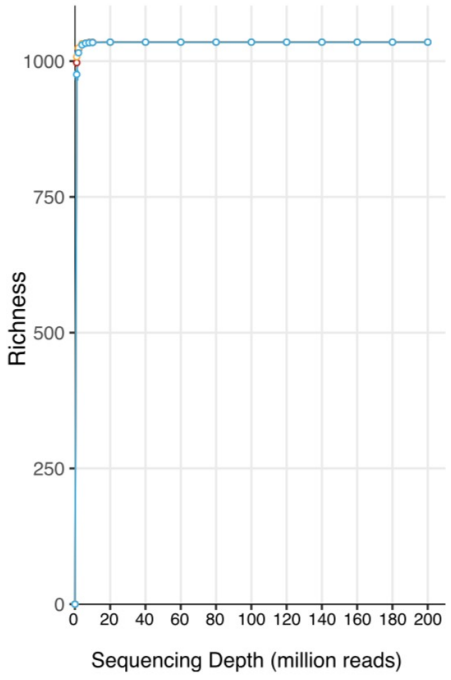
Taxonomic profiling is much more stable to sequencing depth than AMR gene content

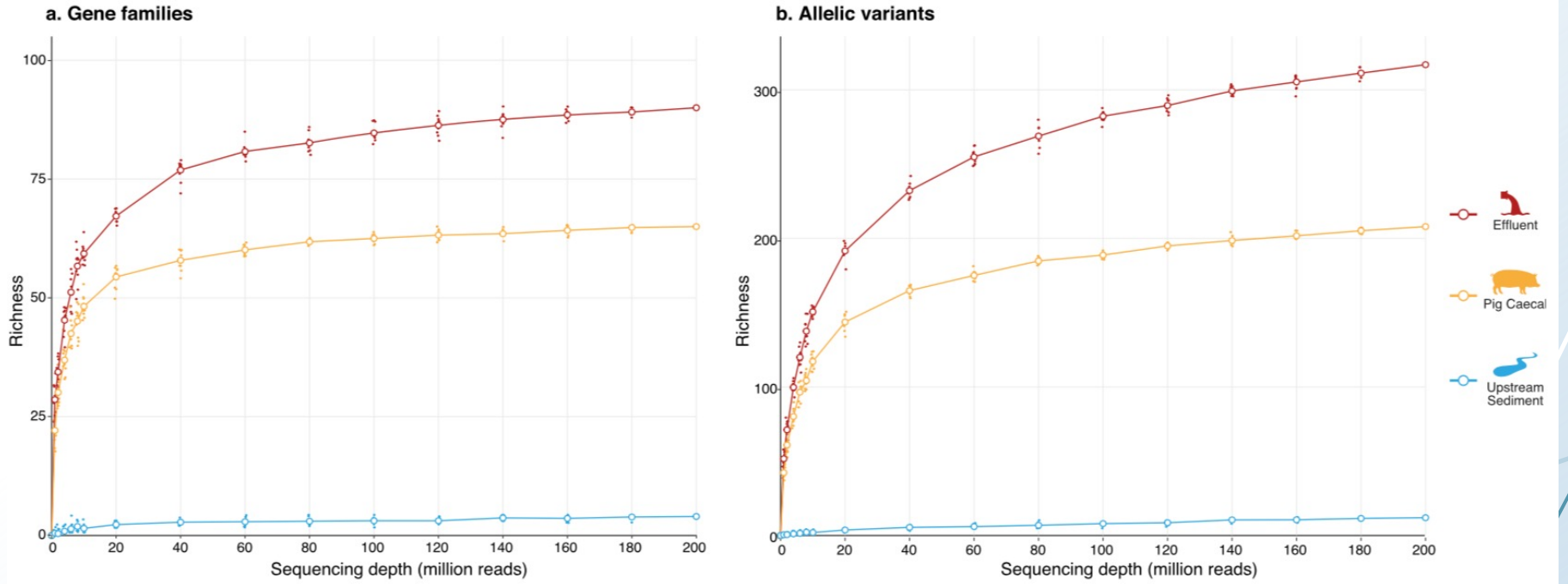
80 million reads per sample were required to recover the full richness of different AMR gene families

RESEARCH ARTICLE Open Access

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

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



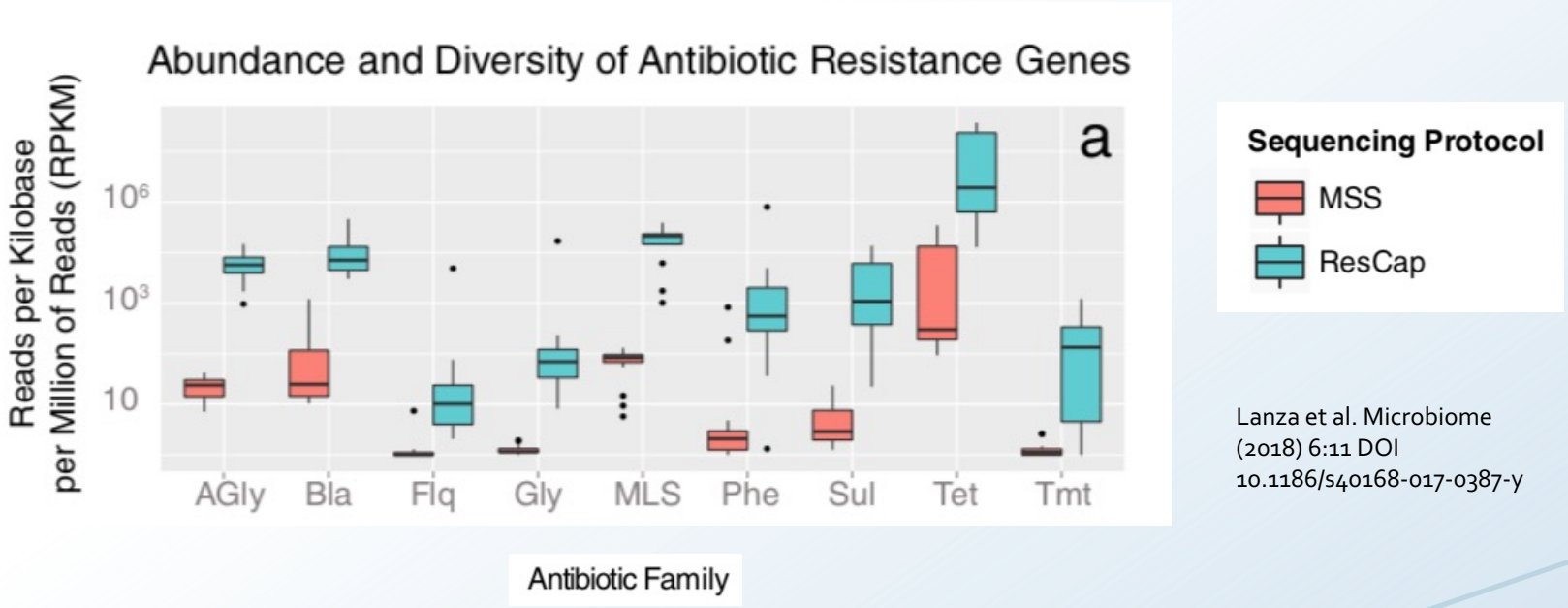


Pros and cons

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 x 10^9 reads per sample to reach a similar coverage to that obtained by using Target capture methods (ResCap)

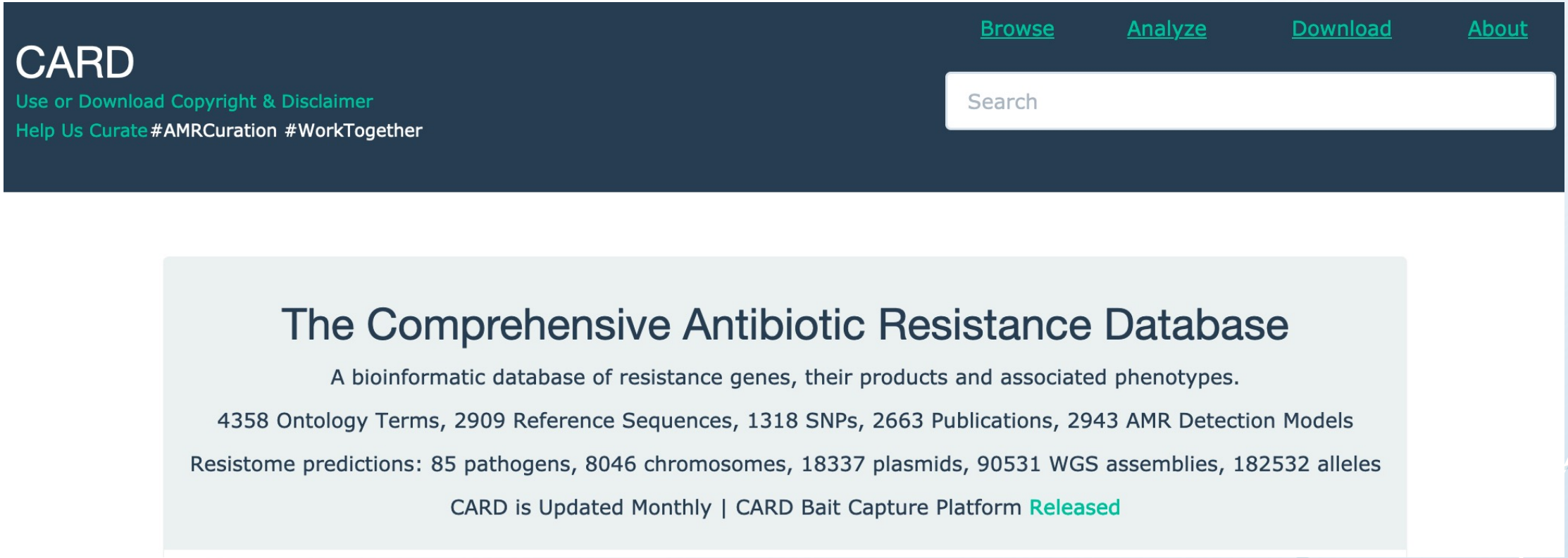


Lanza et al. Microbiome (2018) 6:11 DOI 10.1186/s40168-017-0387-y

Main ARG databases

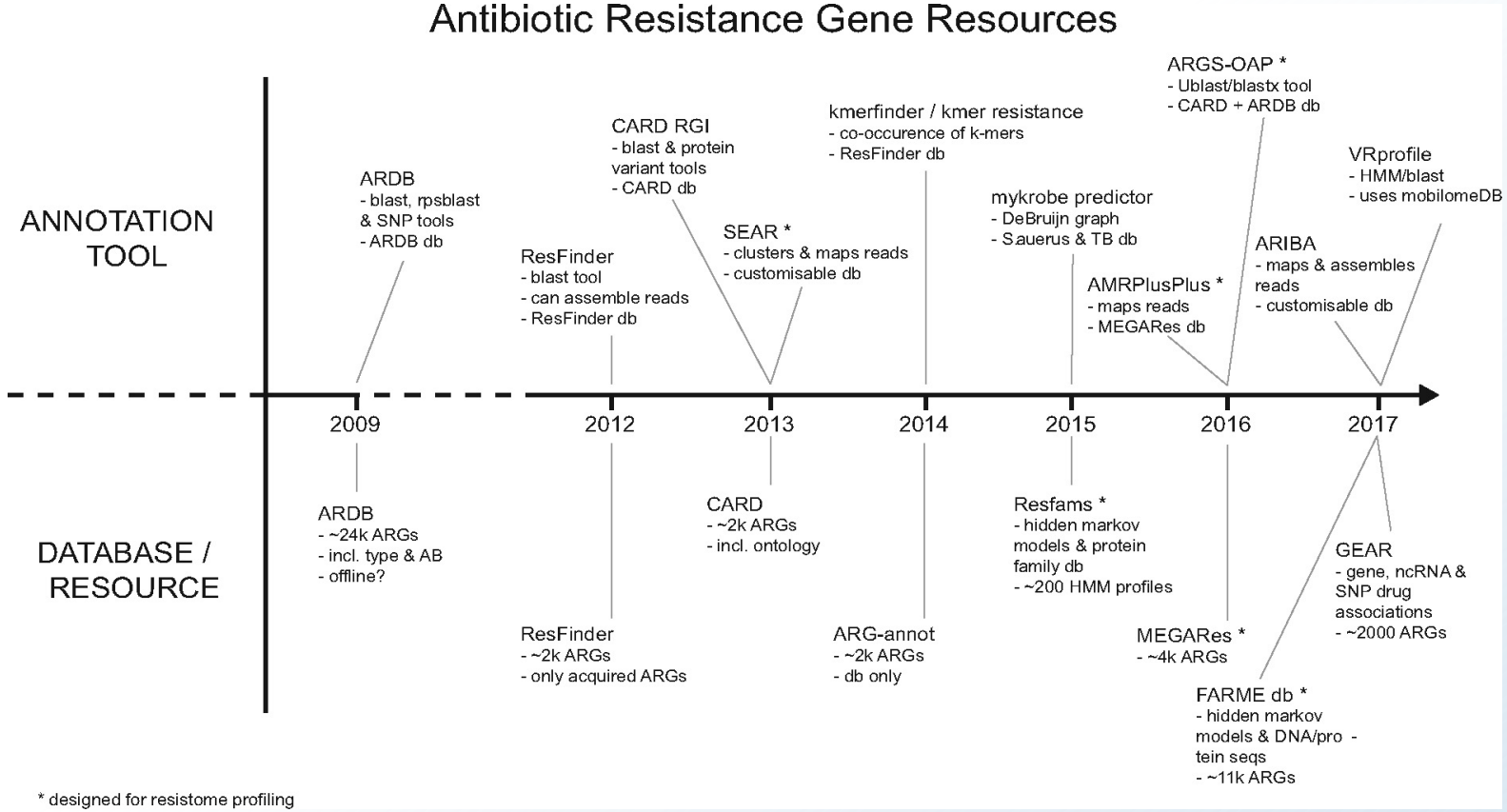
CARD, ARG-ANNOT, ARDB, RED-DB, ResFinder and Resfams

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



Tools to identify ARGs in metagenomic samples by searching ARG databases

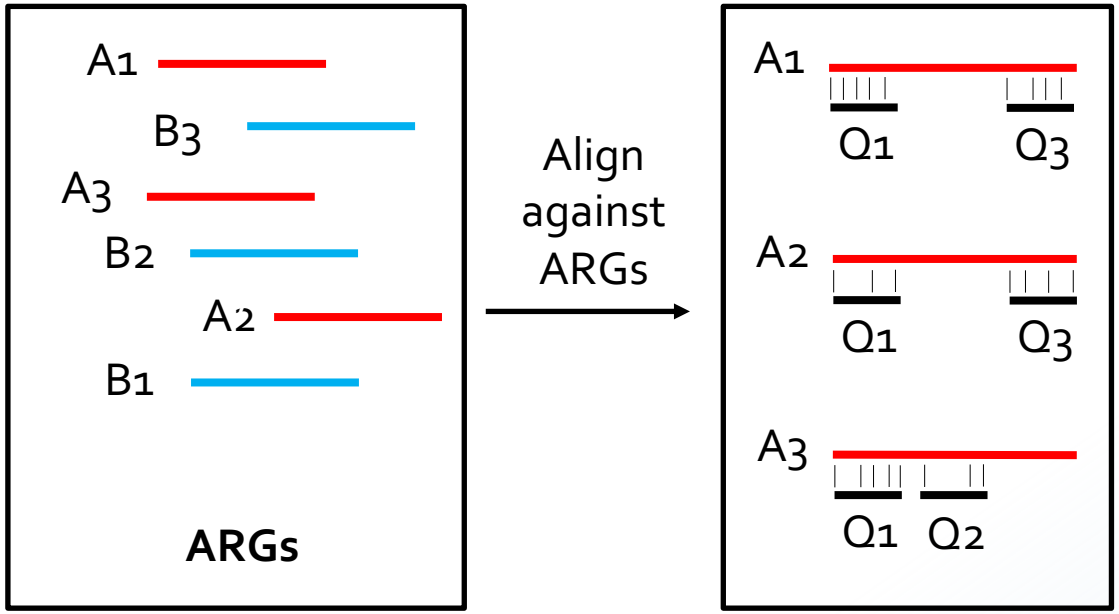
Tools and databases that are used to detect ARGs in sequencing data or assembled contigs

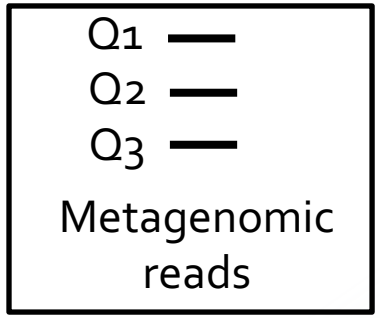


Bioinformatics, Volume 34, Issue 21, 01 November 2018, Pages 3601–3608, https://doi.org/10.1093/bioinformatics/bty387

Querying an AGR database - Homology search (Simplest method)

High similarity shared between reference sequences





Disadvantages

Ambiguous alignments

Unaligned reads (false negatives)

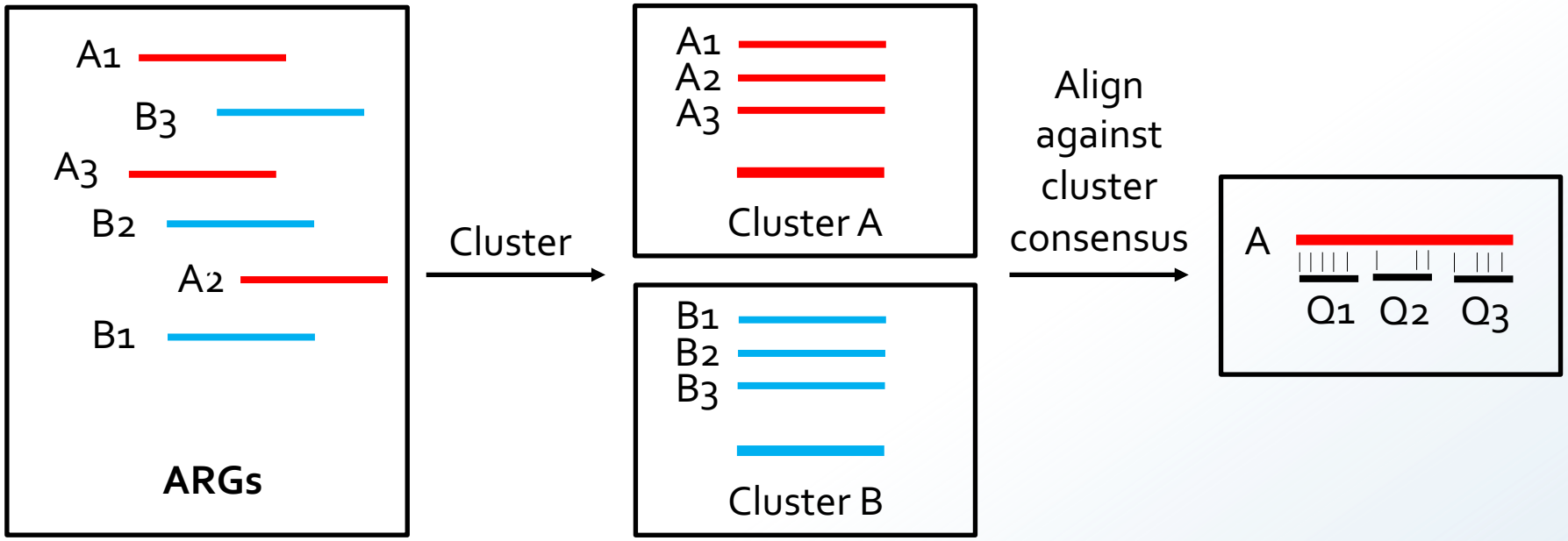
Mis-annotated reads (false positives)

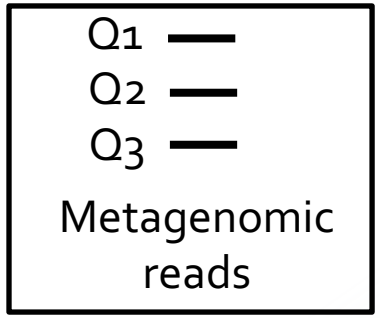
=

Reduced accuracy when typing ARGs

Querying an AGR database - Cluster similar AGRs

The cluster consensus sequences are targets for read alignments





Disadvantages

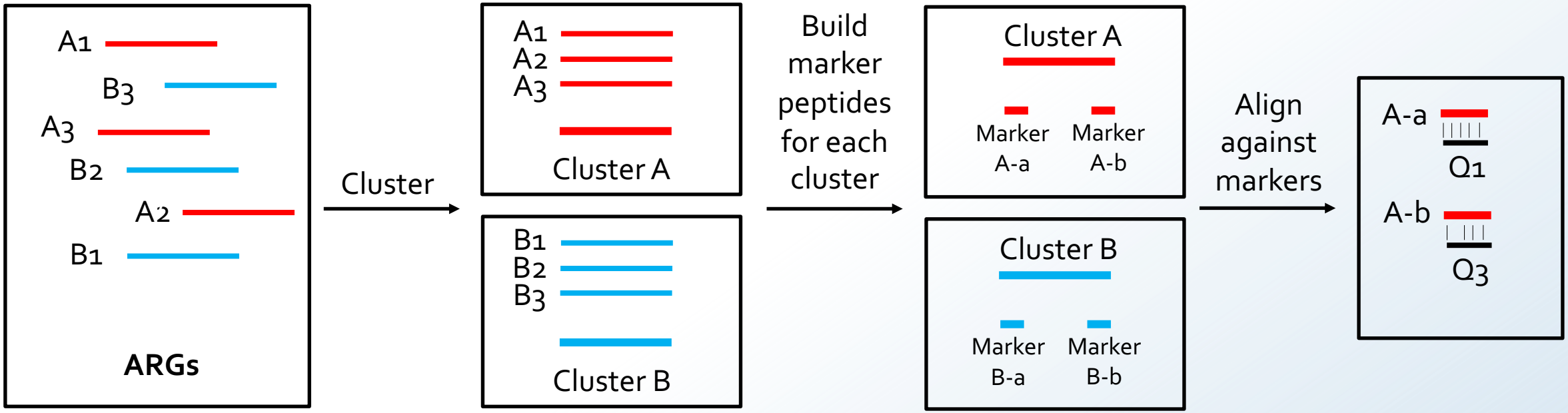
Loss of information as ARG subtypes will be masked

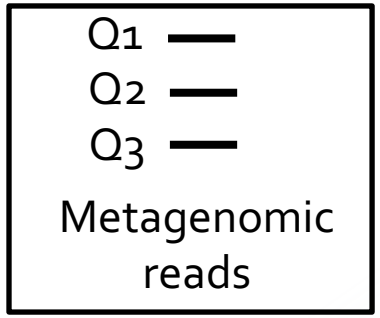
=

Reduced accuracy when typing ARGs

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

Markers represent a family of ARGs





Disadvantages

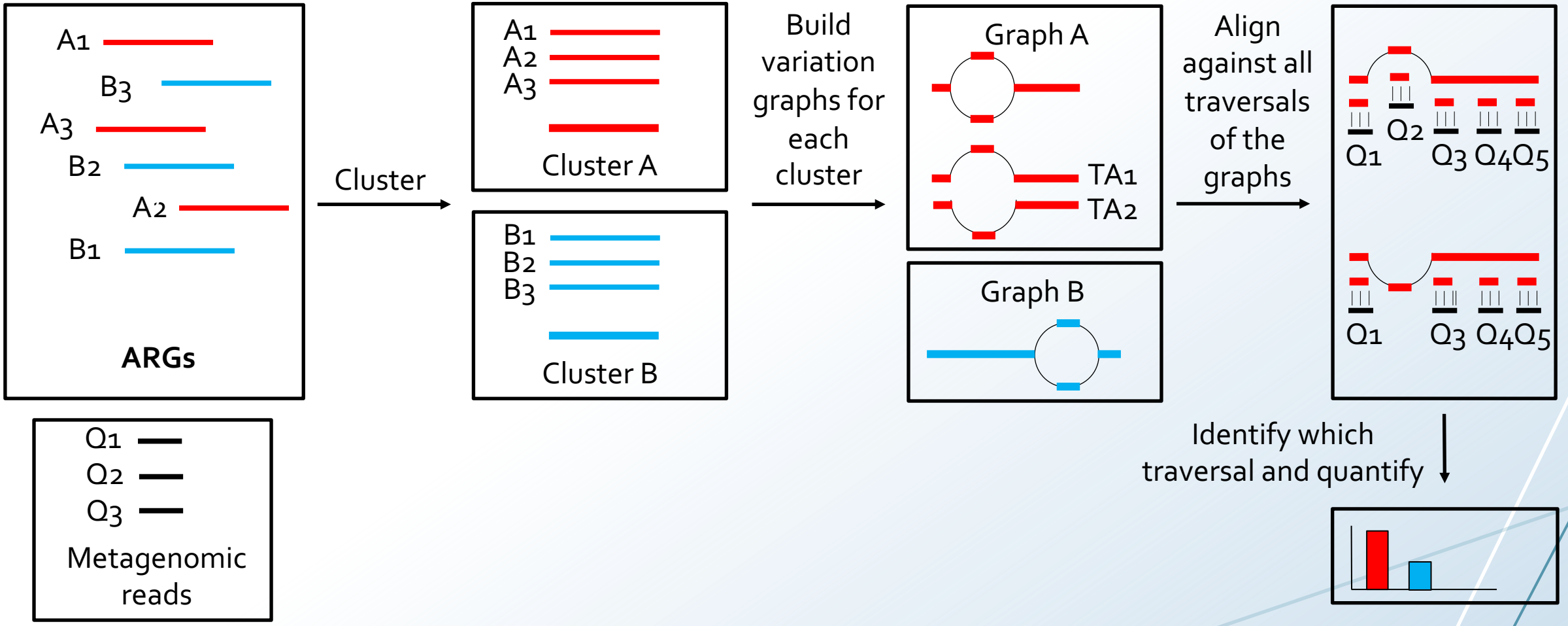
Loss of information as ARG subtypes will be masked

=

Reduced accuracy when typing ARGs

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

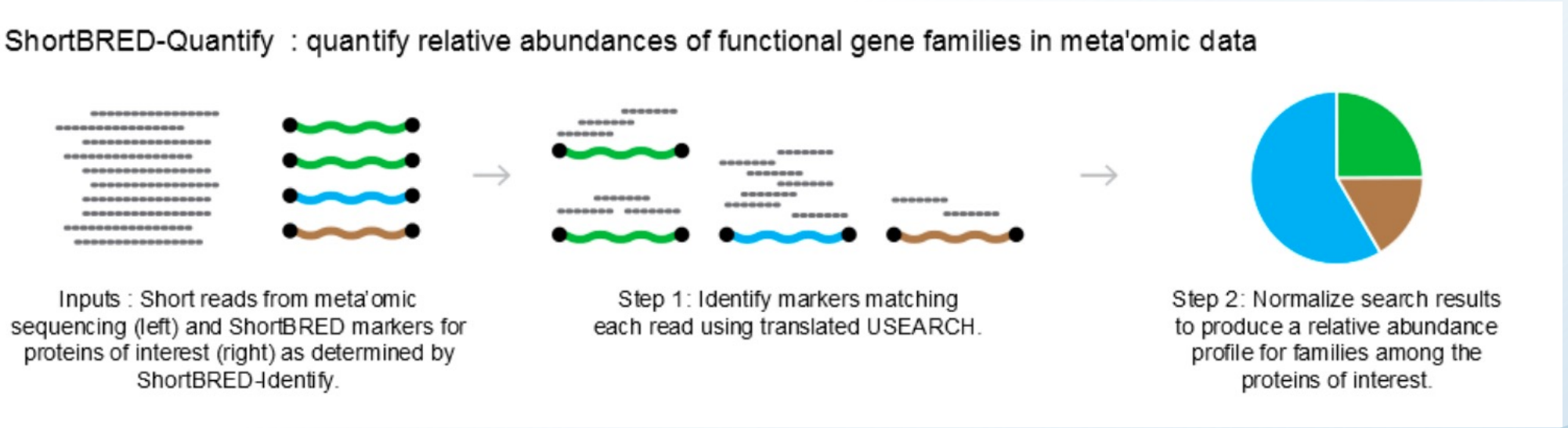
Variation graph represent an ARG cluster and retain sequence variations within a gene cluster



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



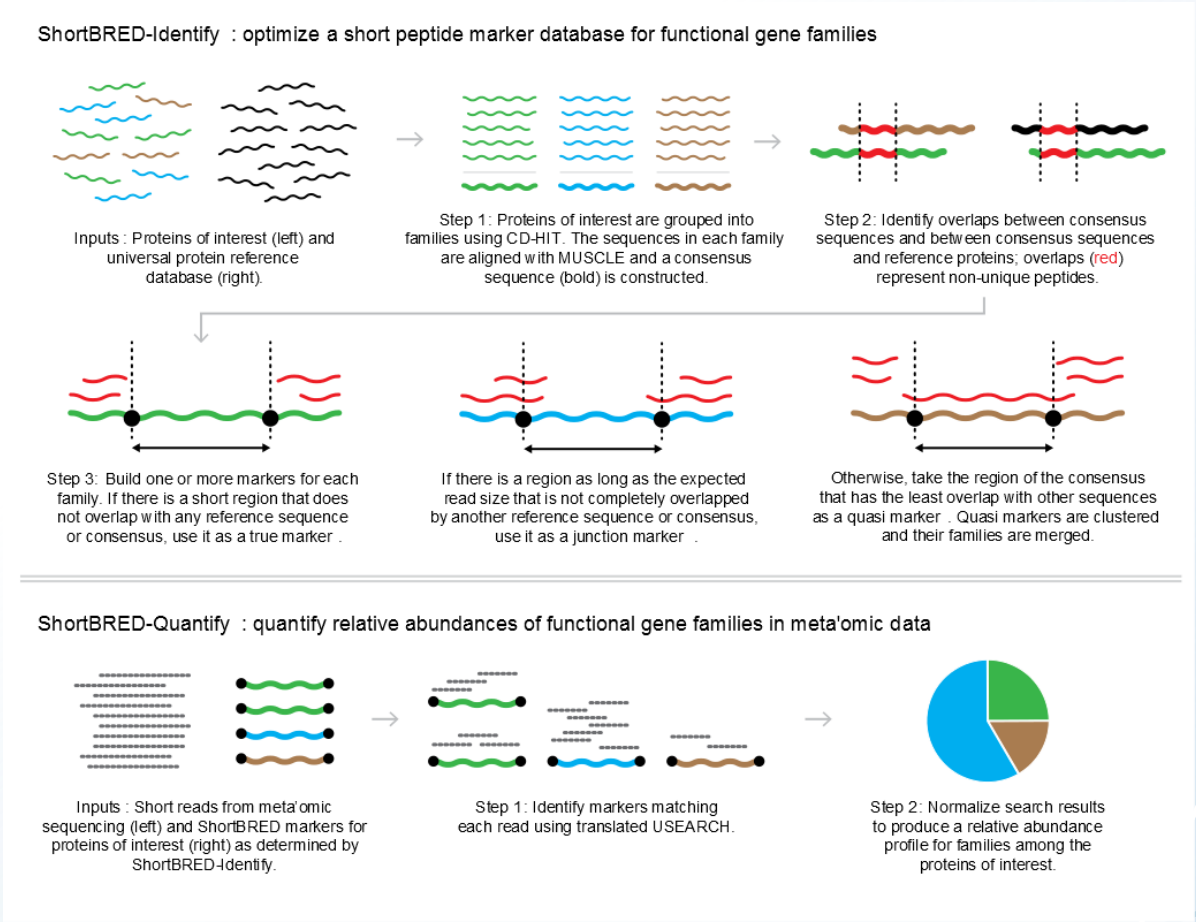
ShortBRED: Short, Better Representative Extract Dataset

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.

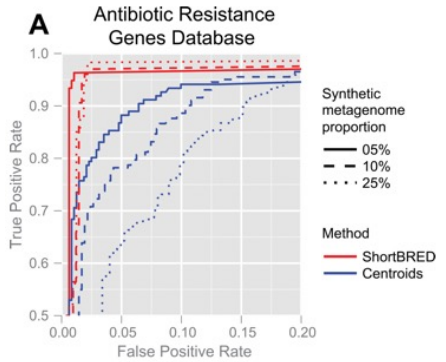
ShortBRED comes with pre-indexed databases

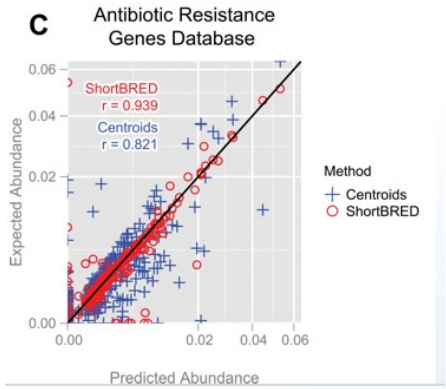


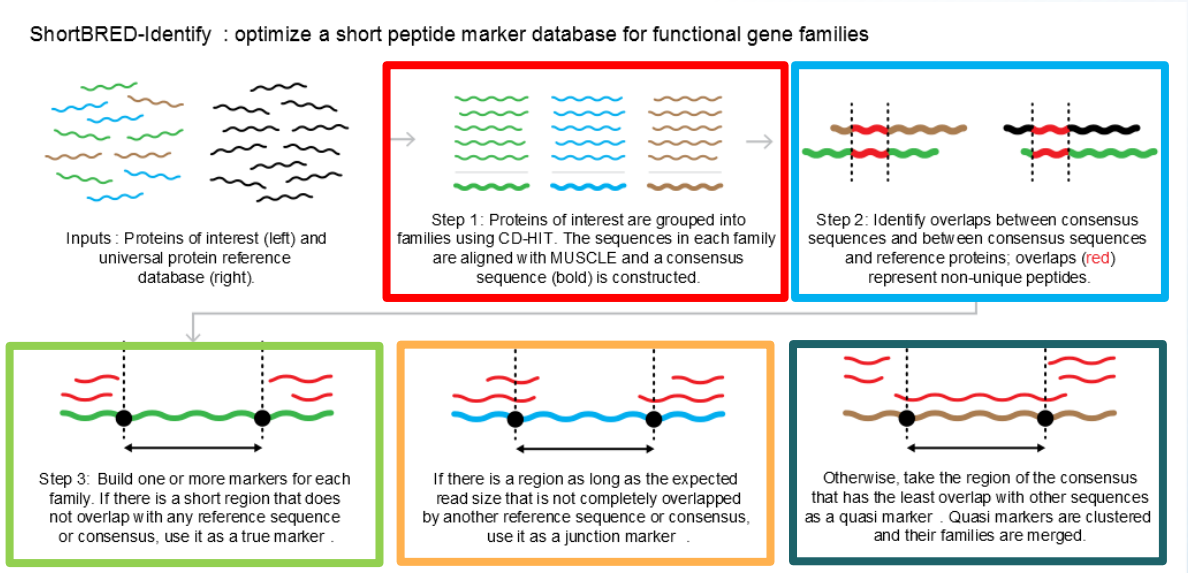
ShortBRED: Short, Better Representative Extract Dataset

Generating markers for the ARDB

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



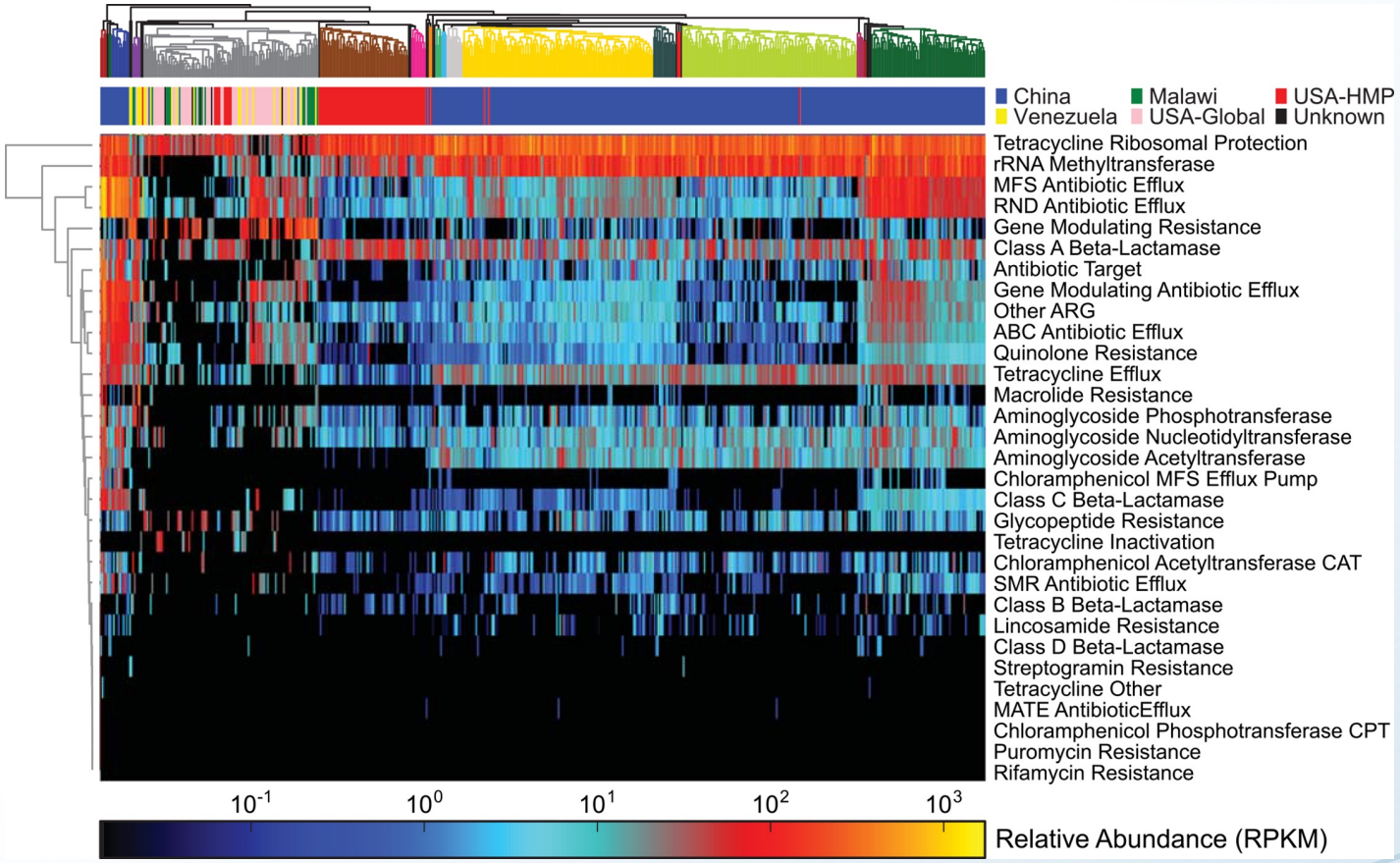




	ARDB
Families after initial clustering	618
Families with true markers	594
Families without true markers	24
Total markers	2,886
True markers	2,845
Junction markers	37
Quasi markers	4

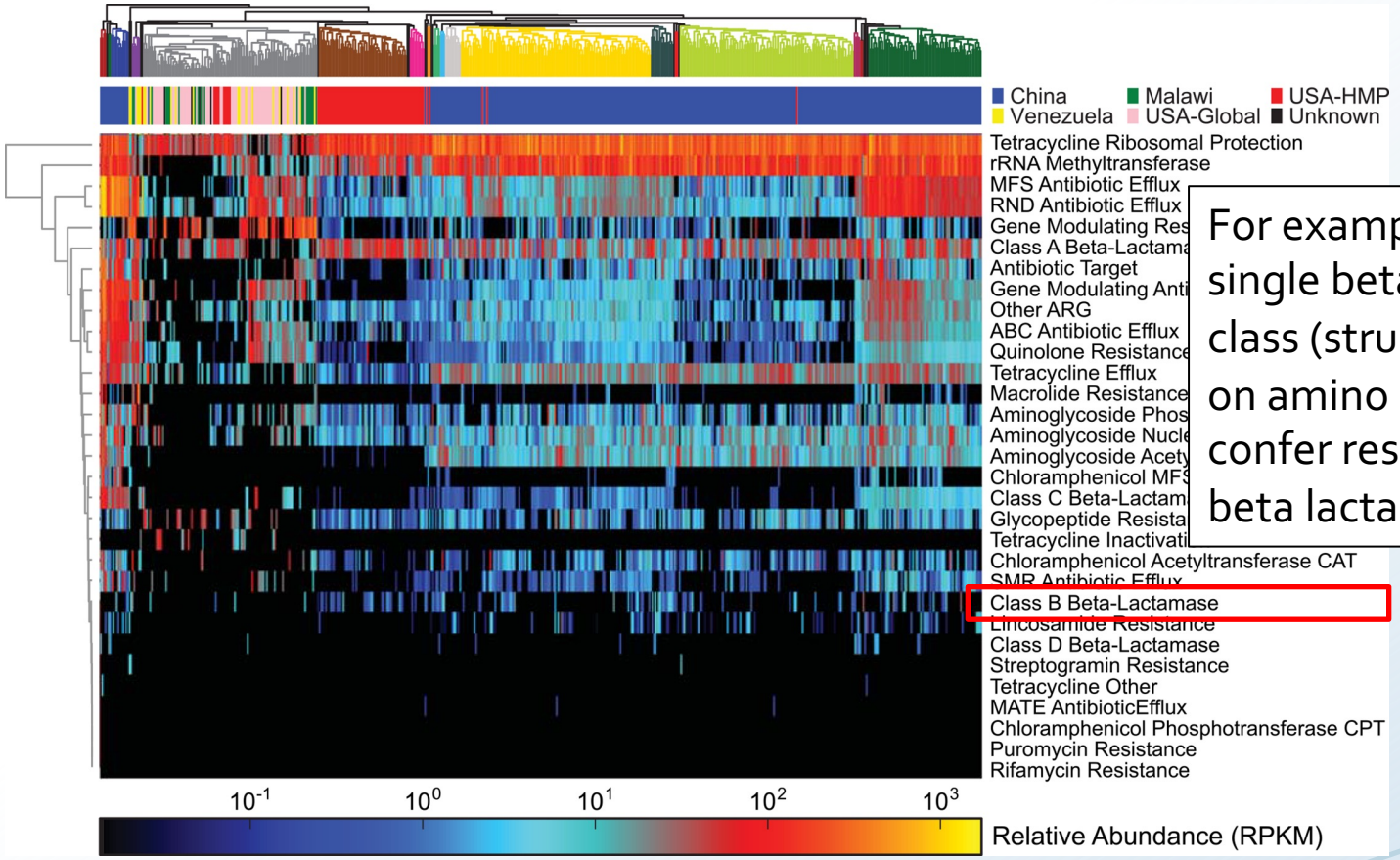
ShortBRED: Short, Better Representative Extract Dataset

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



For example genes within a single beta lactamase gene class (structural classes, based on amino acid sequence) can confer resistance to different beta lactam antibiotics

GROOT - graphing Resistance Out Of meTagenomes

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