PUBLICATION NOTE

Targeting the "Resistome": Sequencing Antimicrobial Resistance Genes with myBaits® DNA-Seq Capture

INTRODUCTION

Profiling the genetic content of emerging resistant pathogens is essential to improve surveillance and monitor trends in antimicrobial resistance (AMR). While total DNA sequencing is a valuable discovery tool, it becomes much more resource-intensive and less sensitive for assessing highly complex metagenomic samples. Alternative technologies such as PCR or microarrays are sensitive, but are limited by narrow target space, and thus are not suitable for comprehensive applications. To address these constraints, researchers from McMaster University established a hybridization capture-based "resistome" profiling method utilizing the myBaits® target capture system from Daicel Arbor Biosciences (1). By targeting over 2,000 nucleotide sequences associated with antibiotic resistance, this capture-based approach not only produced higher on-target read counts and greater breadth of coverage than shotgun sequencing, but also identified additional makers that were inaccessible to shotgun sequencing alone, thereby enabling comprehensive and sensitive detection of antimicrobial resistance markers in diverse sample environments in which antimicrobial resistance genes only represent less than 0.1% of the metagenome.

IMPORTANCE OF RESISTOME PROFILING

Antimicrobial resistance (AMR), which develops when pathogenic bacteria acquire antimicrobial resistance genes (ARGs), is considered one of the most pressing global public health threats. Overuse of antimicrobials in modern medicine and agriculture have been driving the significant increase of AMR and accumulation of new ARGs. Worldwide leading health institutes such as the WHO and the CDC have acknowledged that efforts in surveillance and monitoring of the spread of AMR and the emergence of new ARGs are essential to combat the global resistance infection crisis. One effective strategy is to comprehensively and accurately profile the "resistome" (the collection of the entire ARGs in a specific bacteria or ecological niche) in emerging resistant pathogens, microbiome and other complex environmental settings such as wastewater, sediment, hospitals, and more.

TECHNICAL CHALLENGE

PCR assays and microarrays are popular tools to detect ARGs, however, these highly targeted assays usually only aim to detect a limited set of AMR markers and generally cannot account for ARG variants and/or newly emerged novel ARGs. The more comprehensive whole metagenome 'shotgun' sequencing approach has made the resistome profiling feasible and accessible. However, in metagenomes, where resistance determinants are in low abundance, excessive deep sequencing of millions of reads is usually required. Although it is considered a workable solution, the high sequencing cost and low detection sensitivity are restrictive for routine use in AMR research and surveillance.

"We wanted a method that could be comprehensive for thousands of genes, and cost-effective and sensitive for deep coverage on the target. " - A. Guitor and colleagues

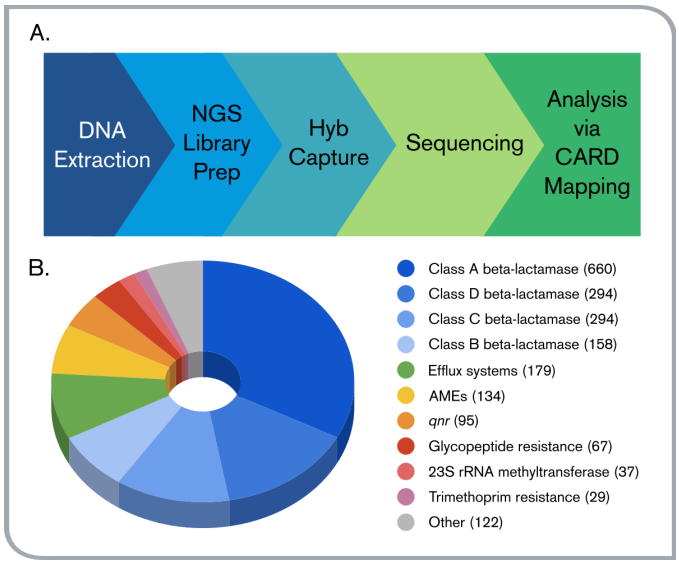

Figure 1. (A.) The schematic overview of the myBaits hybridization capture workflow and identification of diverse ARGs; (B.) Breakdown of the CARD annotated resistance gene classes of the 2,021 targeted AMR markers.

THE SOLUTION

A more comprehensive approach for resistome profiling in metagenomes is to implement a hybridization capture-based strategy (Fig. 1A). To assess the utility of this strategy, the research team selected Daicel Arbor Biosciences to produce a myBaits® Custom target capture kit comprising a total of 37,826 probes to specifically target over 2,000 nucleotide sequences associated with antibiotic resistance (Fig. 1B).

"We chose the myBaits hybridization capture platform for our project because of the flexibility in selecting our targets, the large capacity in the sequence target range, and the clarity and ease of use of the protocol. And the Arbor team has always been supportive of our research projects and are quick to respond with their expert advice and experience using this platform." - A. Guitor and colleagues

RESULTS

Guitor and colleagues conducted a series of experiments using the myBaits capture system and shotgun sequencing on "mock metagenomes" (pools of multiple bacteria). An average of 90% enriched reads mapped to the targeted regions compared to 1% average for shotgun sequencing (Fig. 2).

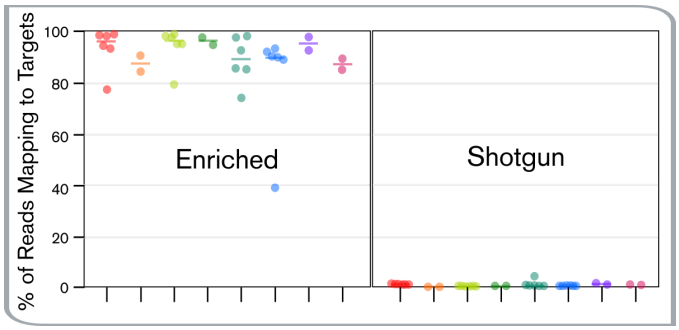

Figure 2. Comparison of percentages of reads mapping to various targets for enriched and shotgun libraries of mock bacterial communities.

To assess the performance in more complex samples, the research team performed similar experiments on replicates from a human gut metagenome. The performance of the capture-based approach exceeded that of shotgun sequencing by achieving >600-fold enrichment, obtaining much higher read count per gene, and identifying more unique antibiotic resistance genes at much lower sequencing depths (Fig. 3).

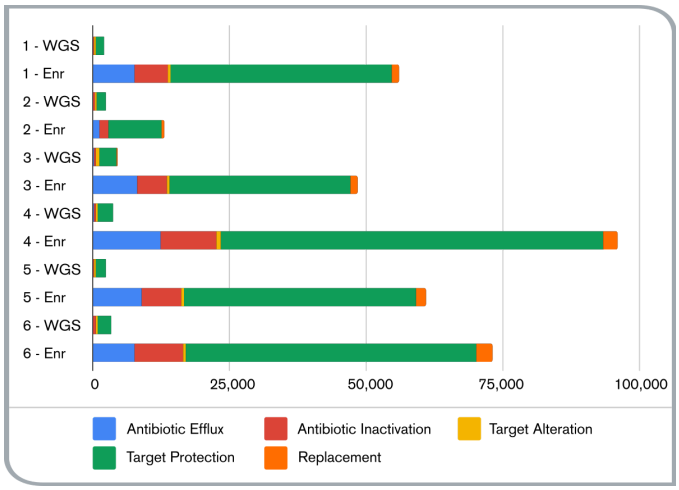

Figure 3. Comparison of read counts summarized per CARD resistance mechanism for 6 human gut metagenome samples between shotgun ('WGS') and enriched ('enr') libraries.

"Using myBaits, we have been able to selectively enrich genes of interest in a high-throughput fashion for a reduced cost compared to shotgun sequencing and obtained more informative sequencing information related to the targeted genes. Without myBaits, it would have not been possible to analyze as many samples as we have in our research given the sequencing cost and computational requirements associated with other methods." - A. Guitor and colleagues

CONCLUSION

By utilizing the myBaits capture system, Guitor and colleagues have established an accurate and sensitive method to profile the resistome and demonstrated the outstanding performance of this method in complex metagenomic samples.

"The custom myBaits probes can be used to profile ARGs in any environment including wastewater, soil, and animal microbiomes. The scalability and flexibility of the probe design with the myBaits platform ensures that we can update our bait set as new ARGs are identified, but that we could also expand to other targets such as unique identifiers of bacterial pathogens or virulence genes." - A. Guitor and colleagues

REFERENCES

1. Guitor, AK. et al. (2019) **Capturing the resistome: a targeted capture method to reveal antibiotic resistance determinants in metagenomes**. *Antimicrobial Agents and Chemotherapy*.

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