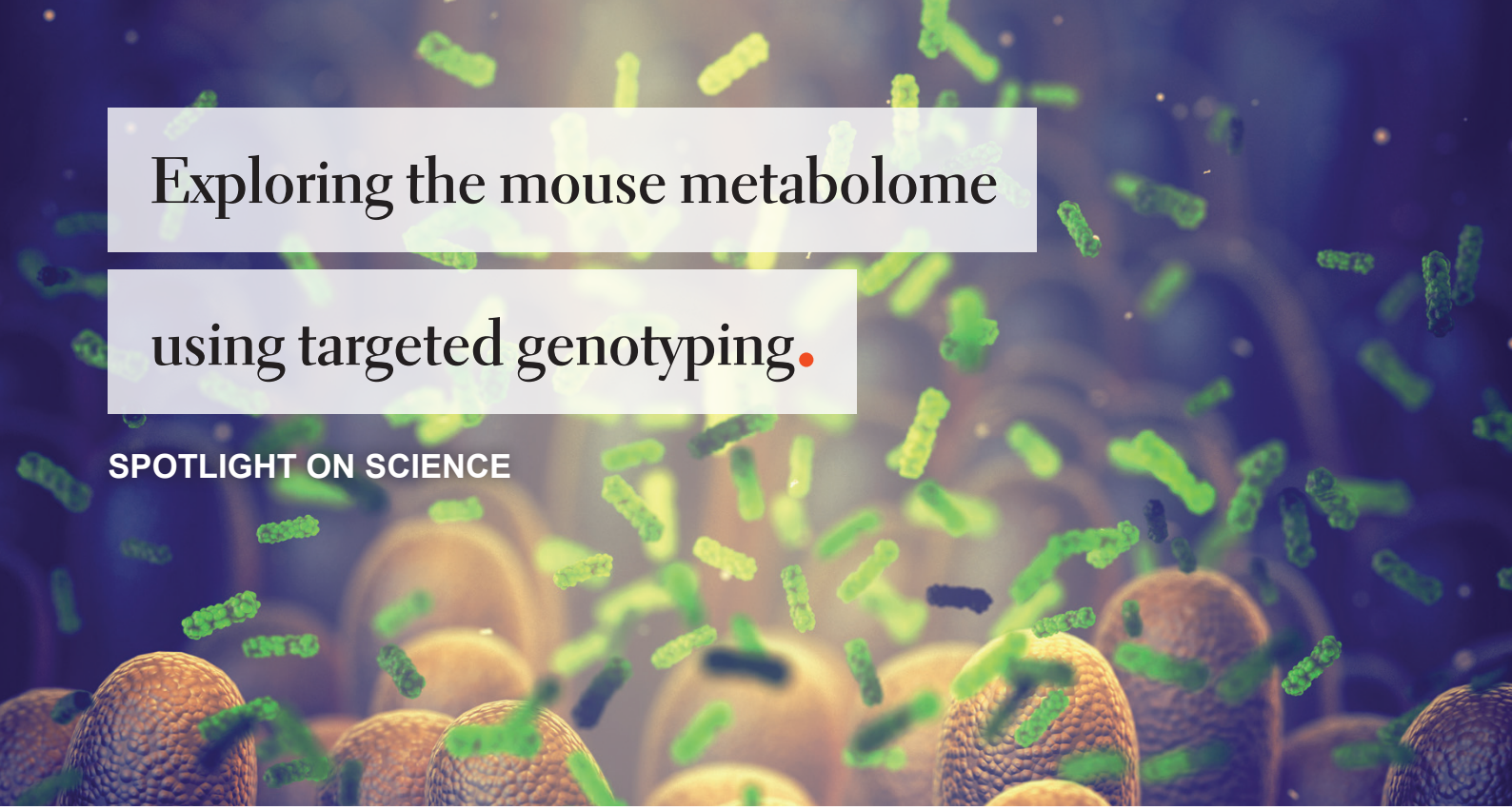

Exploring the mouse metabolome using targeted genotyping.

SPOTLIGHT ON SCIENCE

Jacquelynn Benjamino
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Bacteria that colonize the gut of animals and humans play vital functions in health and disease, but despite this, scientists have only just begun to skim the surface of the microbiome. Researchers in The Adams lab of The Jackson Laboratory for Genomic Medicine are focusing on the creation of different targeted assays and analyses to help identify gut bacteria that lead to disease, using mice as their model organism. They have successfully applied Allegro® Targeted Genotyping, coupled with the Allegro probe design method, to create an assay that targets multiple bacterial genomes in a community. These methods can provide insights into the role of the microbiome in health and disease, for the purpose of diagnostics and treatment.

The interest in the mouse microbiome

The Jackson Laboratory for Genomic Medicine, Connecticut, is dedicated to discovering the complex causes of human disease, and its Adams lab is developing new targeted assays for sequencing potential disease-causing bacteria. To date, there has been a lot of work conducted on the human microbiome to characterize and culture gut bacteria creating an extensive reference database, however, the mouse microbiome is still largely unexplored, despite being one of the most prolific model organisms used in research. “One of the aims of my work is to expand microbiome research for the mouse model, with the eventual goal of translating our research for use in human studies,” explained postdoctoral researcher Jacquelynn Benjamino.

Developing a new assay

Interest and research into the microbiome have boomed in recent years, and it is important to explore new, refined methods for sequencing to advance the field of genomics. The commonly used technologies – including 16S rRNA sequencing and shotgun metagenomic sequencing – are convenient and widely accepted, but present their own limitations, such as bias towards certain organisms when short sequences are used in 16S sequencing. The Adams lab is developing a genotyping method using probes to identify specific organisms in a community and get a more precise understanding of the bacteria present in the mouse microbiome. Jacquelynn continued: “We wanted to use a more targeted approach than traditional methods, and develop our own padlock probes, but were having difficulties. We spoke to another Jackson Laboratory group that was already using Allegro Targeted Genotyping and, although this

assay would normally be used for SNP analysis on one genome, we thought that we could use the same concept to target multiple bacterial genomes in a system."

Exploring the mouse microbiome

The Allegro Targeted Genotyping method was used to generate specific probes for identifying 830 mouse gut bacterial genomes. This efficient technology allows the thorough interrogation of up to 100,000 SNPs in one assay, by combining three key technologies: enzymatic fragmentation, ligation and targeted genotyping using single primer enrichment technology (SPET). The researchers used their own bioinformatics pipeline to narrow down a pool of over 80,000 potential probes to around 16,000 – almost 20 probes per genome – by screening for cross-hybridization to other parts of the same genome, or any of the other 829 genomes. They also worked with Tecan's bioinformaticians on developing probes using the Allegro probe design method – Tecan's own solution for labs without extensive bioinformatics capabilities – and found both methods to be equally valuable for creating technical replicates of probe pools.

The Allegro assay improved the efficiency and intensity of the work-flow as Jacquelynn explained: "The protocol was so easy to follow, everything was outlined well, and it was great to simply have it work straight away. Another thing that is great about the Allegro assay is the ability to pool samples. By ligating each sample to one of 48 unique barcodes, then pooling up to 48 samples into one tube, you're working with far fewer samples for the remainder of the protocol. For my work, this took 192 samples down to just four tubes after one step!"

The potential to scale up

With the scalability and flexibility of this method, it could have far wider applications for the identification of bacteria. "While this assay is currently for use in the mouse model, it is easily transferable to other models by creating a suitable probe set. I think this approach holds enormous potential for general community surveys, or for tracking a certain pathogen in a system. If you want to scale up to 1,000 or 5,000 genomes, you're going to need a lot of probes, but this is achievable with the Allegro Targeted Genotyping assay. It will be exciting to see how our work progresses using it, and what applications it may have in the future," concluded Jacquelynn.

To find out more about Allegro Targeted Genotyping, visit **www.nugen.com/products/allegro-targeted-genotyping-v2**

To learn more about The Adams Lab, go to **www.jax.org/research-and-faculty/research-labs/the-adams-lab.**

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