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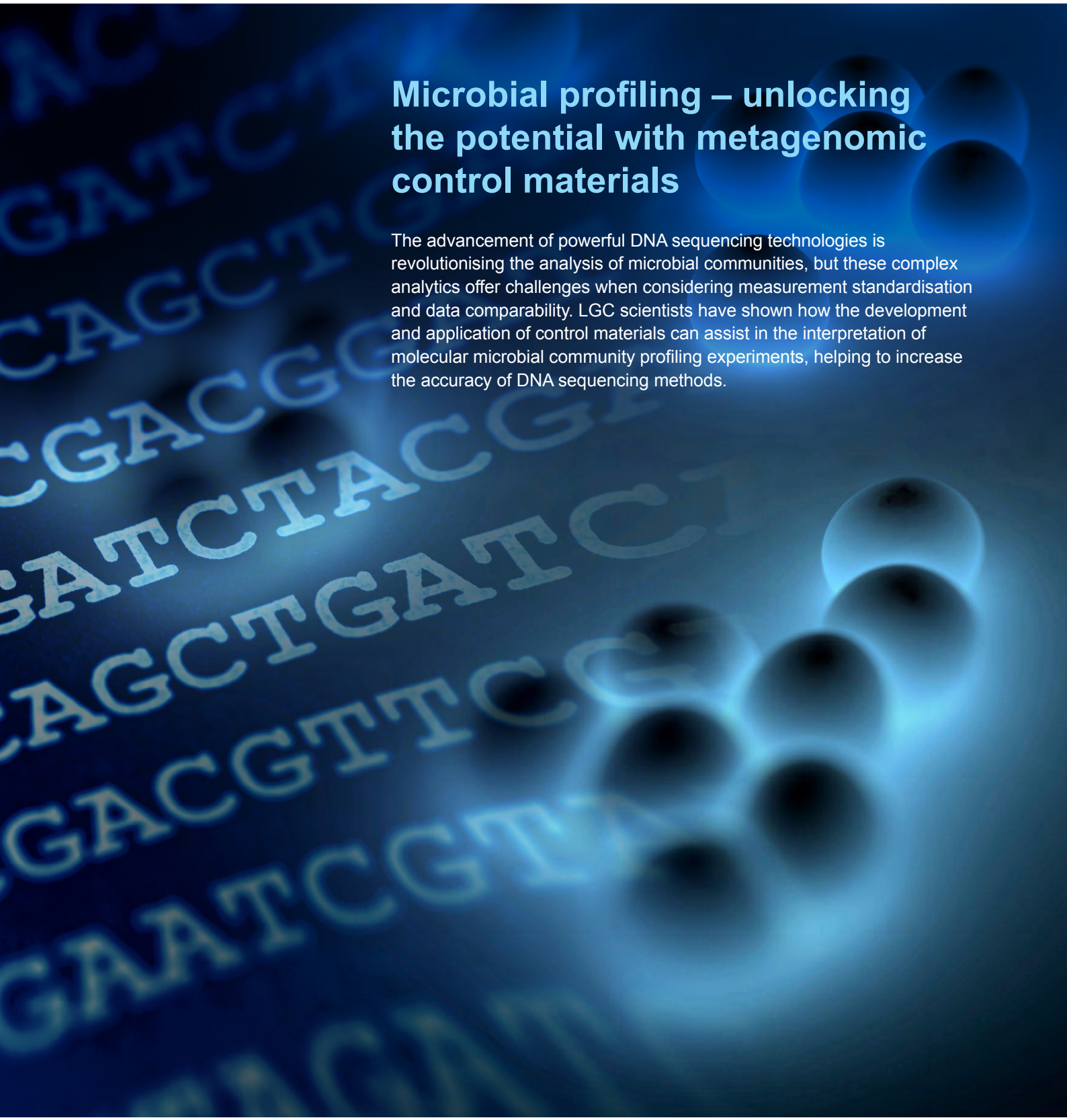

Microbial profiling – unlocking the potential with metagenomic control materials

The advancement of powerful DNA sequencing technologies is revolutionising the analysis of microbial communities, but these complex analytics offer challenges when considering measurement standardisation and data comparability. LGC scientists have shown how the development and application of control materials can assist in the interpretation of molecular microbial community profiling experiments, helping to increase the accuracy of DNA sequencing methods.

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The requirement

The analysis of the genetic material of a microbial community is called metagenomics and can provide valuable information about the environment from where it is sampled. Understanding the microbiological population dynamics has a wide range of applications in the industrial, clinical, environmental and agricultural fields.

Humans, for example, carry tens of billions of bacteria that frequently perform important roles, from protecting us from attacking microorganisms to assisting with our digestion, and vastly outnumber the harmful microbes that can cause illness. While humans have around 24,000 genes in their genome, it is estimated that the genetic material from bacteria living symbiotically with us contain over 360 times more, which presents a significant analytical challenge.

High throughput DNA sequencing techniques, such as Next Generation Sequencing (NGS), can enable scientists to study tens of thousands of microbial genomes in a single run. These techniques have revolutionised the analysis of genetic information. However, this revolution presents new analytical challenges, due in part to the complexity and size of the resultant data sets. NGS also requires a complex and multistep preparation procedure with a variety of potential adaptations which can all result in measurement bias. NGS experiments are also expensive which can limit experimental replication. These factors, plus the lack of suitable reference materials, present challenges for the standardisation of metagenomic measurements and the comparison of experimental results.

Metagenomic control materials (MCM) are required to enable laboratories to test their experimental procedures for bias, and to evaluate the precision of their data.

The solution

LGC scientists, in collaboration with the University of Exeter, have identified and developed standards and controls needed to aid metagenomic analysis. A prototype MCM comprising a mixture of ten different bacterial pathogens has been prepared. The bacterial DNA was mixed at defined proportions based on mass. The concentrations were chosen to reflect an approximation of a real clinical extract. This material was then used to assess different sequencing approaches.

In an initial study the prototype material was analysed using two versions of the same sequencing method which had very subtle differences. The results showed that while one of the methods produced results that were close to the expected values, the other showed considerable bias for some of the pathogens. The use of the MCM demonstrates that findings can differ considerably depending on the initial experiment set-up and illustrates the value of control materials as an essential tool for assay development and application.

Impact

The human microbiome market is expected to reach $658 million by 2023 with therapeutics expected to be the largest proportion of the market by 2019. By 2018 the global market size for probiotics is projected to be $37.9 billion. Comparison to a known sample provides crucial support for method development and validation which is essential for the development of these markets. Accurate and comparable metagenomic approaches will facilitate the impact of associated research, ensuring that our understanding of the microbial world in the context of the environment and human health is not misguided by technical bias.

Following the publication of the findings [1,2], Dr Vladimir Benes, Head of Gene Core at the European Molecular Biology Laboratory (EMBL), commented:

> *"Thank you for sharing your findings. The subtle technical differences highlighted by the control material clearly have the potential to dramatically affect metagenomic quantification, and consequently influence conclusions drawn from the data. At the EMBL Gene Core facility we will certainly make our users aware of your findings to inform their experimental design. It appears the application of this type of control material could greatly assist our understanding of how to perform accurate metagenomic microbial community profiling."*

[1] J. F. Huggett, T. Laver, S. Tamisak, G. Nixon, D. M. O'Sullivan, R. Elaswarapu, D. J. Studholme, C. A. Foy, "Considerations for the development and application of control materials to improve metagenomic microbial community profiling", Accred. Qual. Assur., 2013, 18 (2), 77-83.

[2] D. M. O'Sullivan, T. Laver, S. Temisak, N. Redshaw, K. A. Harris, C. A. Foy, D. J. Studholme, J. F. Huggett, "Assessing the accuracy of quantitative molecular microbial profiling", Int. J Mol. Sci., 2014, 15(11), 21476-91.

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4381/CB/0515