The Importance of Microbiome Characterization in Research Reproducibility

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TransnetYX® Microbiome

The microbiome is a fundamental regulator of phenotype

The gut microbiome is composed of a rich collection of microbes that regulate the immune and nervous systems, digestion, metabolism, and drug responses.[1-8] While the mechanisms of action continue to be established, the influence of the gut microbiome across such a diverse range of host systems can alter and confound phenotypes. **Numerous studies have demonstrated that changes in the microbiome can cause phenotypic differences within an experiment or across experiments, and that this may lead to the misinterpretation of results.**[9-10]

The microbiome is markedly distinct in animals from different commercial vendors, with further variance caused by background strain and genotype. However, unlike genetics, **the microbiome remains dynamic and can shift as a result of any change in the environment**. For example, even subtle variations in the diet, cage type, cage location, housing density, and bedding type can significantly impact microbial composition.[11] Drugs commonly used for the regulation of conditional models, such as doxycycline, tetracycline, and tamoxifen, can modulate the microbiome within an experiment.[12-13] In addition, the transportation of animals can be a stressful process, affecting hormone levels and eating and drinking frequency. This change can transiently affect the microbiome during the transfer of animals to a new institution, or even between facilities at the same institution.[14-16]

In order to maximize experimental reproducibility across the scientific community, a growing body of research demonstrates that microbiome analysis and monitoring is a critical part of a comprehensive animal characterization protocol that reinforces the scientific rigor of investigations.

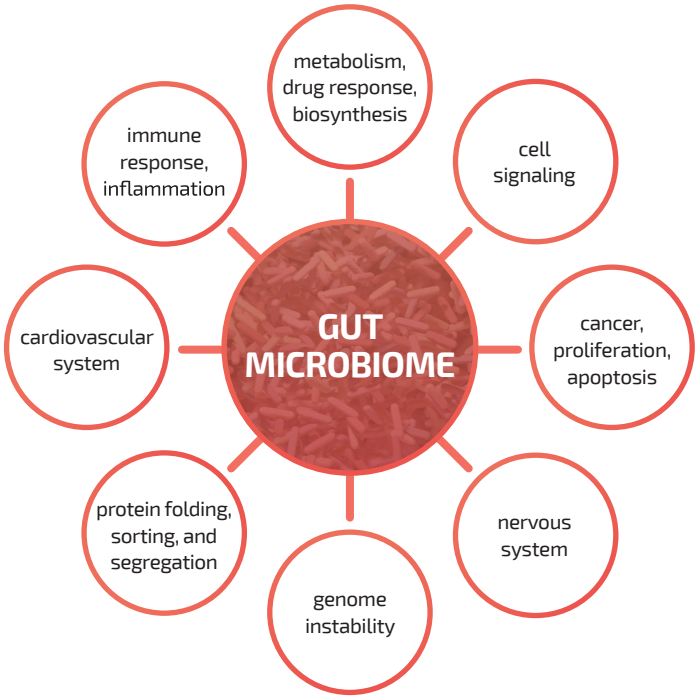

Figure 1. The gut microbiome regulates a wide range of host systems; thus, compositional shifts can directly impact experimental phenotypes.

The evolution of animal characterization in research reproducibility

Laboratory animal models remain invaluable to biomedical research, with direct links to pivotal discoveries that have enriched human and animal health. Yet, the quality and reproducibility of data produced by laboratory animal studies has improved over the last 70 years through strides in the characterization of these models.

Until the 1960s, most commercially available rodent colonies were infected with pathogenic or potentially pathogenic bacteria, mycoplasmas, parasites, and murine viruses.[17] The development of gnotobiotic, germ-free mice by Philip Trexler and James Reyniers at the University of Notre Dame paved the way for the development of specific pathogen-free (SPF) animals, which lack certain pathogens known to afflict conventional colonies.[18-20] These two advancements ultimately allowed the production of disease-free laboratory animals for biomedical research.[21] These improvements in rodent health profiles enhanced the consistency of experimental data, making the characterization and monitoring of animal health a milestone in promoting animal research reproducibility.

The evolution of animal characterization in research reproducibility (continued)

The rapid proliferation of genetically defined rodent colonies quickly demanded another level of characterization beyond colony health status. Outbred, inbred, hybrid, congenic, spontaneous mutant, and genetically modified animal models are subject to changes in phenotype based on integrity of the background model and genotype. Early last decade, The International Council for Laboratory Animal Science (ICLAS) developed the program "ICLAS Network for Promotion of Animal Quality in Research" that specifically calls for the development of a genetic monitoring program on an international scale. The program aims to assure the reproducibility of experimental results by using genetically defined animals, which, like all defined reagents used in an experiment, can directly impact results. Using poorly defined animals can render research data meaningless or unrepeatable due genetic contamination, making the analysis of animal genetics a paramount precursor to their use.[22]

The advancement of sequencing technologies has enabled the investigation of the gut microbiome with unprecedented resolution and throughput with substantial reductions in cost.[23] The development of these data has allowed a deeper understanding of the role and impact of the gut microbiome to animal health and disease. It has become increasingly evident that changes or disruptions to the gut microbiome can have significant effects on animal models and their expressed phenotypes, adding a complex and important variable into basic research and pre-clinical studies.[24] An increasing number of publications define the loss, gain, or shift in expressed phenotype concurrent with a change in the animal microbiome, often as a result of the influence of microbes on the host immune, metabolic, or nervous system responses. As a result, microbiome monitoring is quickly becoming a third essential objective in the characterization of research models.

Developing a microbiome monitoring program

Because of the varying nature of the microbiome, the decision of when to analyze microbiome composition is critical. A regular, scheduled surveillance program is optimal and can make microbial shifts in an animal or colony more apparent. While the associated costs can sometimes seem unfeasible for smaller labs, the cost of repeating experiments due to confounded phenotypes is often much more substantial. While it can be tempting to collect and store samples to be analyzed at a later time pending a clear change in phenotype, this can be too late and result in the loss of precious time, animals, reagents, and money. There are specific points at which microbiome analysis can be particularly helpful[25]:

- When ensuring the microbiome is not impacting or confounding the results of an experiment (pre- and post-study), particularly when the experimental protocol has the potential to disrupt the gut microbiome
- When acquiring animals from a different vendor, institution, or lab to ensure the microbiome has stabilized
- When creating a new strain
- When introducing a new breeder
- When proactively monitoring or purposefully making changes to the environment of the animals (water, diet, bedding, room)

Complexities of microbiome analysis methodologies

While the microbiome research field rapidly expanded, experimental best practices were slower to develop. The collection of samples themselves can be complicated, as collection should be completed under sterile conditions to avoid microbial growth that could impact the sample profile. Historically, samples have had to be immediately frozen and shipped on dry ice to ensure preservation. However, the development of stabilization buffers allows for a simplified protocol, with samples being collected, stored, and shipped at ambient temperature without alterations in microbial composition.

Furthermore, the method of DNA extraction can also significantly impact the recovered microbial profile. Different protocols can bias results due to enhanced or reduced ability to recover microbes based upon their size or structure.[26-27] Commonly used methods of sequencing, such as 16S rRNA sequencing rely on sequencing only a single component of a single prokaryotic gene to determine microbial composition. This method is immensely dependent upon the primer sequence chosen and is limited to the analysis of bacteria at a family or genus level. Updated methodologies, such as shotgun metagenomics, leverage Next Generation Sequencing at a shallow depth to elucidate the complete diversity of the microbiome, providing insight into fungi, viruses, archaea, and protists, in addition to bacteria. This method provides species, strain, and often substrain-level taxonomic resolution without the substantial costs normally associated with deep sequencing.

Data analysis and interpretation

Historically, sequencing has required dedicated biostatisticians to even begin analysis. However, modern bioinformatic software can provide user friendly, automated methods of data visualization with built-in statistical analyses. These interfaces can make data interpretation more approachable for users outside of the field of sequencing, while also allowing seamless data sharing with present or future collaborators.

These platforms can make the observation of microbial shifts over time more apparent. For example, fluctuations in alpha diversity (microbial diversity within a sample) and relative abundance can be easily observed using an area plot view with samples from different time points. A shift in the gut microbiome and a concurrent change in research results suggest that further investigation is warranted. Were there changes in husbandry, such as feed or water? Was there an experimental manipulation that might drive a change in the gut microbiome? Just as we might not understand the underlying molecular pathways for a shift in phenotype associated with contamination of background strain in a mouse model, we do not need to fully understand causation in a shift in phenotype associated with a change in gut microbiome of the research model.

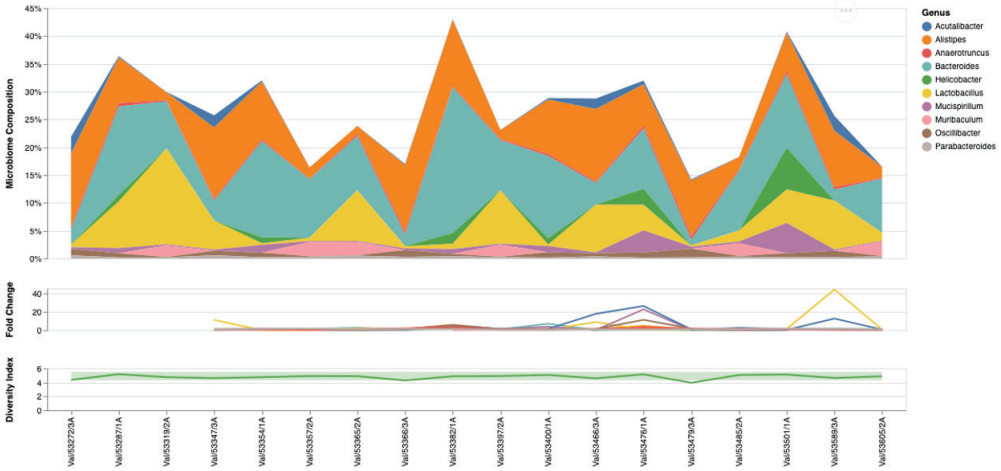

Figure 2. Modern software can Modern software can automatically create area plot views of microbiome composition, allowing immediate insight into sample composition and changes over time. Advanced features, such as the diversity index in Transnetyx BiotaBase™, quantify sample diversity and compare it to the ranges of diversity observed for all processed samples.

Data analysis and interpretation (continued)

Establishing a baseline microbiome profile for a colony facilitates the ability to pinpoint when changes in abundance and diversity happen. As more microbiome data is collected and shared using standardized protocols, the definition of "normal" for microbiome composition becomes more apparent. For instance, the BiotaBase™ software from Transnetyx uses data from the ongoing collection of thousands of samples to generate a diversity index. This index identifies the observed ranges for sample diversity to alert users when a sample is outside the standard bounds. By retaining fecal pellets, an animal or colony microbiome can also be restored and refreshed, if necessary.[28]

Conclusion

Microbiome shifts can cause unexpected effects upon research and animal phenotypes.
The monitoring of microbiome composition is an integral part of modern, high-quality scientific research and facility management. Surveying colonies routinely can ensure that changes that impact research outcomes are quickly identified, and the loss of valuable time and resources across labs and institutions is minimized.

For more information on Microbiome Analysis and surveillance services
Ready to get started? Transnetyx offers a comprehensive Microbiome Analysis service, including sample collection kits, an interactive results interface, and cloud-based data storage to simplify the monitoring and surveillance of your colony's microbiome. To learn more, visit **www.transnetyx.com/microbiome** or contact Transnetyx Genetic Services at +1 888 321 2113.

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