ZYMO RESEARCH

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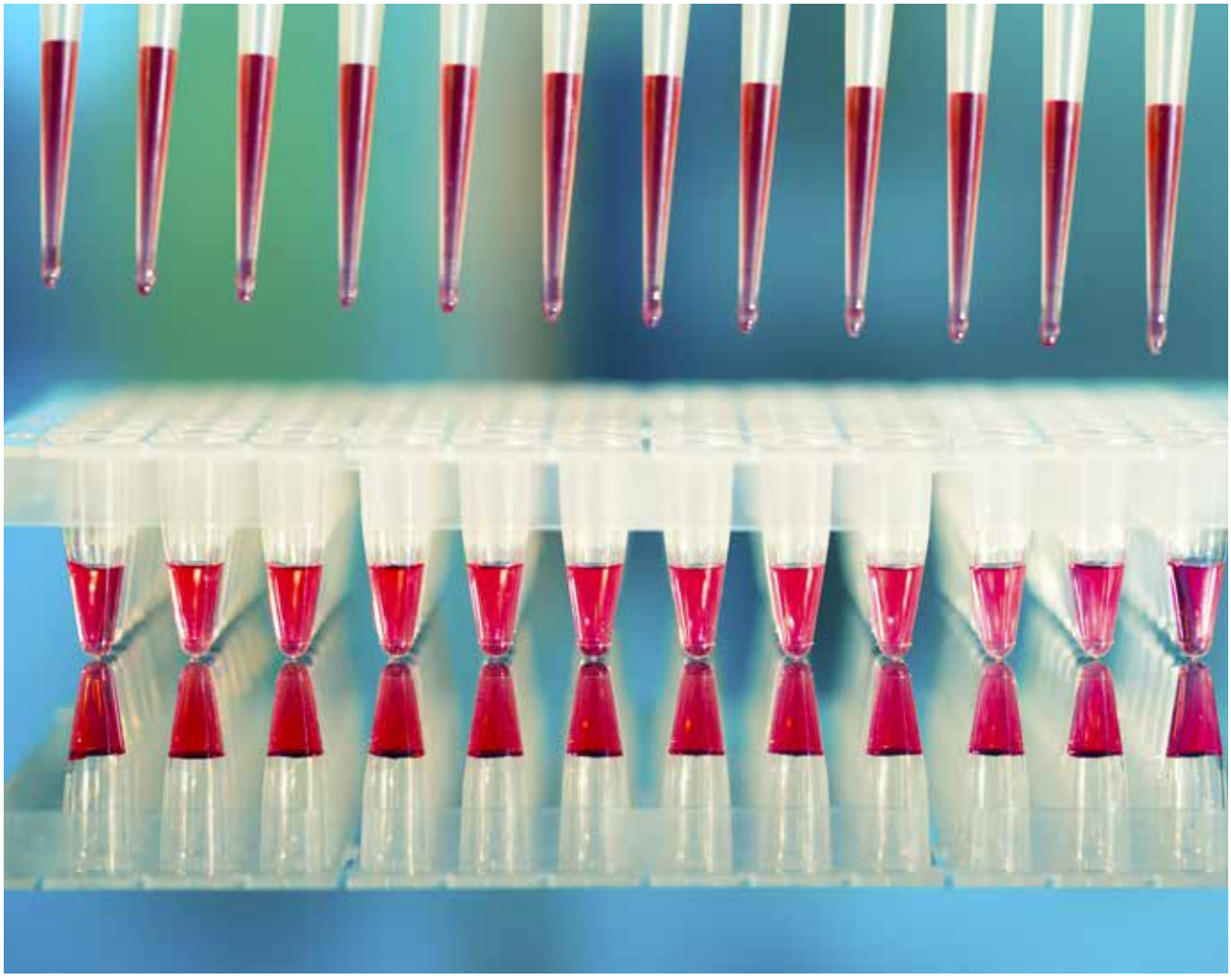
Tips for Biopharma Professionals Designing and Planning a Microbiome Research Study



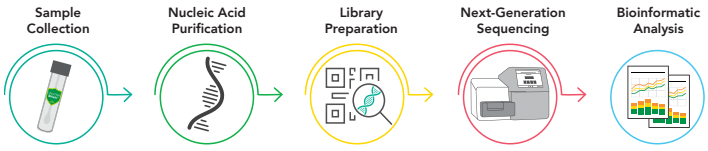
The field of microbiology has experienced a renaissance over the past decade. The recent advances can be attributed in large part to the so-called Next Generation Sequencing (NGS) revolution that has propelled much of genomics research over the same time period. Using the newest and most powerful sequencing technology, scientists are now able to gain access to an unparalleled understanding of the diverse community of trillions of microbes that exist and thrive literally within each of us and our surrounding environment. Thanks to such studies, we are now better able to understand how a healthy microbiome helps to train our immune system, regulate metabolism, influence production of key neurological transmitters like serotonin, aid healthy digestion, and thereby impact our overall health and well-being[1-4].

In some cases, the natural healthy composition of microbes in a given community will fall out of balance, a phenomenon known as dysbiosis, and contribute to disease. Recent studies have demonstrated how dysbiosis contributes to conditions including infectious disease, ulcerative colitis, inflammatory bowel disease, obesity, autoimmunity, neurological disorders, and even cancer[4-7]. As the biomedical community better understands how the microbiome contributes to disease, that knowledge can be applied to the development of improved therapeutic interventions. For example, knowledge of the microbial community composition represents a potentially rich source of biomarkers to study disease association and target engagement. Similarly, scientists are beginning to understand how probiotics & pre-biotics can be implemented to improve diverse health outcomes. The human microbiome is deeply involved with so much of normal human biology that the biopharmaceutical applications from its more thorough understanding are only limited by imagining what might be possible.

Looking beyond human health, microbial communities play an important role in maintaining the natural flora and fauna of the entire planet. As such, the microbiome carries potential for advancing green initiatives and improving the environment. For example, plant biologists study the microbiome to find ways of improving crop yield and feeding a hungry planet when faced with the alternate challenges that flooding and drought pose to the agricultural community. Similarly, research scientists are exploring ways to change the microbiome of ruminating livestock such as cattle to reduce greenhouse gas emissions and thereby combat the rising threat of global warming.

With so much potential to advance biomedical research and the practice of medicine, there are many new biopharma companies, entrepreneurs, innovators, and scientists exploring ways that a microbiome study can help advance their own research goals. Careful planning and consideration for design and conduct of such studies is essential to ensuring data fidelity and long-term success of the study's outcomes. With that in mind, here is a list of 5 key areas to consider when initiating a microbiome study.

1. Sample Collection
2. Nucleic Acid Purification
3. Sample Prep (NGS)
4. Choice of Sequencing Platform
5. Data Analysis



1. Sample Collection

When it comes to researching a microbiome, the capabilities of collection should be the first obstacle to consider. Capturing a snapshot of the microbial community and maintaining the integrity of that initial population distribution are of utmost importance. Careless collection methods can lead to issues like DNA and RNA degradation or shifts in the microbial population of the sample over time which would render future analyses meaningless. To ensure the stability of a sample's microbial profile, traditionally collected samples are immediately frozen to minimize profile bias.

Pathogen inactivation is another major consideration at the sample collection step. Working with microbiome research samples that are potentially pathogenic represents a biohazard, which can place severe restrictions on the scope of any study. However, collection methods that are able to inactivate pathogens at the point of collection can help to relieve such barriers by rendering samples non-pathogenic, non-biohazardous, and non-infectious. In so doing, samples become safe to handle from the start of any study and thus easier to integrate into any downstream molecular analysis workflow.

As 2022 comes to a close, and researchers look ahead to a post-pandemic era, business-to-business and business-to-consumer entities alike have come to realize that globalized supply chains are an increasingly tenuous operational framework. The ability to ship samples or products across borders presents its own set of challenges, particularly if cold-chain shipping is required. It might be necessary to ensure that your lab infrastructure has an international presence in order to complete a project with minimal logistical delays.

2. Nucleic Acid Purification

Another major consideration is the choice of nucleic acid purification and extraction methods used in a study. Sample extraction can be the make-or-break factor for obtaining reliable data. Regardless of which sample type you are investigating (e.g. soil, fecal, vaginal, skin, or oral) a nonbiased extraction method that gives full coverage of microorganisms is critical. Not all extraction techniques are equal as studies demonstrating the differential lysis efficiency of gram-negative versus gram-positive bacteria will attest[8,9].

If purification and extraction methods are not reproducible, the rest of your sequencing and analysis will come into question. Make sure you have a workflow or are working with a provider that thoroughly understands the intricacies of DNA and RNA purification from microbial samples. Using a microbial standard of known composition is an excellent way to add an internal control for bias introduced during extraction.

3. Sample Preparation

More factors come into play prior to the sequencing stage. You did the work and collected your samples properly and have used a reputable extraction method. Now considerations of reproducibility come into play. All your efforts can still be squandered and confidence in the data can be lost if bias is introduced in the next step. Standards need to be included in the NGS sample preparation stage to ensure that sequencing results are accurate and reliable.

Sample traceability and barcoding can also be a factor at this stage of development. If you plan to prepare your samples in-house, lab personnel with the right expertise and the proper equipment are needed to ensure that samples are organized throughout the entire workflow.

Regulatory guidelines may be another consideration for your research. GLP and CLIA certifications may or may not be needed at your stage of development. However, conducting research under a well-structured regulatory framework is always a good idea at any point in a research study.

4. Choice of Sequencing Platform

Now that you have successfully collected samples, isolated the nucleic acid contents, and put together protocols for sample preparation, you will need to select the sequencing platform appropriate to meet your study's goals. Sequencing technology platforms can be fundamentally broken down into two basic categories – either short-read or long-read instruments. The short-read technology marketplace has been dominated for years by industry leader Illumina, Inc. However, upstarts including Singular Genomics, Ultima Genomics, Element Biosciences, and Beijing Genomics Institute (BGI) subsidiary MGI Tech Co. are now vying for market entry as well. By comparison, long-read sequencing platforms

are dominated by two companies, Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT).

Short-read technology excels in producing very large quantities of data at a relatively low price. Application of short-read technology enables research methods collectively known as shotgun metagenomics, which provide high taxonomic resolution of all microbes in a sample as well as the functional roles performed by that microbial community. Although the cost has dropped dramatically in recent years, metagenomic analysis can still be expensive in certain cases. Furthermore, shotgun metagenomic methods can be subject to host contamination, especially for skin, oral, or cancer microbiome studies, which can increase cost and confound data analysis.

By comparison, long-read sequencing technology excels in applications related to sequencing assembly, which are otherwise extremely difficult using short-read technology. Furthermore, long-read technology applied to targeted analysis of the 16S (bacterial), ITS (fungal), or 18S (other eukaryotes) genes allows high taxonomic resolution and detection in samples that are either low in microbial abundance (e.g. marine, soil, or other environmental types) or contain a high host to microbe content ratio (e.g. skin, biofluids). Despite PacBio's and ONT's recent and laudable technical advances made to their flagship platforms, Sequel IIe and PromethION, respectively, questions related to accuracy and throughput compared to the short-read technology remain. However, PacBio's newest HiFi sequencing configuration quells many of those criticisms and points to a bright future for long-read tech.

Along with choosing the appropriate sequencing platform, you need to ensure that your sequencing is completed within a timeframe that algins with your project goals. High throughput processing may also be needed deepening on the scale of testing. If you plan to batch samples and utilize the services of a sequencing provider, make sure they have the capacity to accommodate your study. If you require flexibility in the amount of sequencing per week or month, that is another important consideration. Put a sequencing schedule in place that covers frequency of shipping, quantity of samples, and turnaround time to receiving data.

In summary, both short and long read sequencing platforms have their own relative advantages and disadvantages. Fortunately, the technology continues to improve year upon year allowing scientists to carry out increasingly more powerful studies. With so many options the most important consideration left is selecting the platform best suited to your research.

5. Data Analysis

You may or may not need full bioinformatics analysis with interactive data reporting but having the option might be something to consider. Carefully assess your personal business model and what exactly you are trying to provide to the final stakeholder. Raw data might be enough for your model, but if you need custom reporting and graphs, you will need an in-house team of experts who have the technical skill to analyze the data. Hiring a qualified group of bioinformaticians can be another struggle, or you may need to find an external provider for this service.

If you decide that outsourcing bioinformatics analysis is the best option for your project, consider how you would like to receive the final data. Cybersecurity is critical when sending or receiving potentially sensitive information. Transmitting data back and forth over email may no longer be sufficient to guarantee the safety of your data. Make sure that your data analysis provider is up to date with the current best practices in the area of cybersecurity.

The Solution

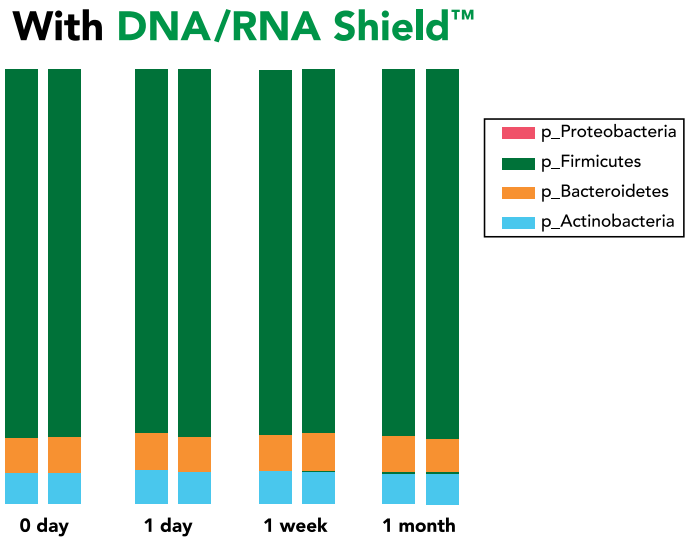
Kickstarting a microbiome research study may seem daunting. Considering all the factors that will increase risk and effect timeliness can be overwhelming. Understanding that any mistake can be costly and cause a product to fail can add to a sense of anxiety. Hiring knowledgeable

employees for each step of the development process can not only be time consuming but will require teams to be created around each aspect of the development process.

There is a way to mitigate risk and set up your efforts for success. Finding a qualified service provider and aligning your research goals with a proven workflow can ensure time, energy, and money are not wasted.

Zymo Research understands the complexity of Biopharma projects and can provide you with the solutions you need for the entirety of your workflow. You can ensure your sample collection is complete with a wide array of collection devices for any sample type. Zymo Research also provides a solution to pathogen inactivation and makes cold chain shipping obsolete with DNA/RNA ShieldTM preservation reagent, which not only stabilizes nucleic acids at the point of sample collection but also inactivates pathogens upon addition. With multiple sequencing labs in the United States, Europe, and South America Zymo Research also simplifies any international shipping or study design complexities.

Microbiome profile preservation at ambient temperature



No significant changes in a microbiome profile are seen over a 1-month timeframe when samples are stored in DNA/RNA Shield™ reagent.

As an industry expert in standards and extraction, you can feel confident that your services with Zymo Research utilize verified and industry leading research methods. Instead of working with multiple providers to help carry out your research, a single source with capabilities to satisfy every step in your microbiome research study simplifies outsourcing and saves time and money in the long run. Zymo Research can also provide you with a variety of sequencing options and an automation team available for high-throughput projects.

To simplify matters further, Zymo Research has an experienced team of bioinformaticians who can work with you directly to provide custom data solutions that fit your research needs. Powered by AWS, data security is covered, and branded reporting options can be created. Comprehensive solutions ensure a smooth research study from a single partner who can work with you and your team throughout the process.

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