Sample Preparation in Microbiome Analysis

Towards Standardized Workflows

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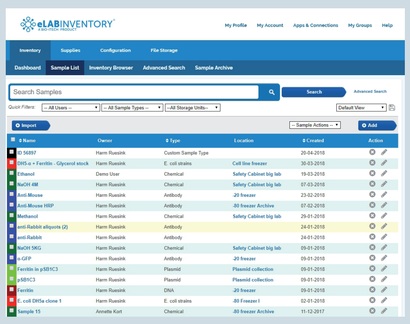
Introduction

Scientific questions around the microbiome such as case – control and longitudinal studies require best practices and reproducible laboratory workflows to process hundreds, sometimes thousands of samples for the identification and comparison of microbial community structure, composition, and genetics, as well as functional variation.[1] Additional considerations in this context are sample collection and storage. Overall it is crucial that microbiome analysis is reproducible.
Next-generation (NGS) sequencing based methods for marker gene studies, metagenomics and metatranscriptomics survey microbial communities with a different focus and detail. Standardization of the NGS workflow from sample collection and storage over sample preparation to insights of the sequencing data is important in order to compare and combine separate studies in consortium based approaches at a global scale. Here, we show the semi-automated liquid handling supported workflows for some of these methods. Some of them find their application in well-validated protocols used with diverse sample sets.[2]
Cultures of the component microbes will allow for a holistic study of microbes and microbial communities and will ultimately allow to guide medical practice as the importance of the influence of the gut microbiome ecosystem in the maintenance of health is increasingly understood.[3]

Eppendorf standard

Sample storage
It is crucial for microbiome analysis to avoid variation during sample collection and shipping, and to allow for storage at -80 °C.



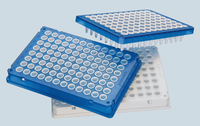
Eppendorf ULT Freezer
> Temperature stability
> Easy integration with sample management software (eLABinventory)
> Advanced interface for sample monitoring

Sample extraction and library preparation
Standardized technical factors and sample processing are essential to control for variation. Depending on the experimental question, samples can contain different concentrations of microbes, can be heavily contaminated with relic DNA, and require special processes. Accordingly, the usage of established extraction and library procedures are important.

ep*Motion*® 5075t
> Industry leading number of methods on a benchtop automate
> Intuitive, powerful software

ep*Motion* 5073m
> Small-scale extraction and NGS library preparation

Mastercycler® X50 PCR cycler
> Reduced sample loss and high yields due to high-pressure lid
> Excellent temperature regulation for consistency
> Increased throughput capacity



LoBind Tubes and Plates / twin.tec® PCR Plates
> Reduced sample loss due to binding to surface
> Optimized fit with magnets, cyclers and ep*Motion*
> Uniform well geometry and rigid design
> No deformation after cycling

Biomass production
The use of microbial communities for healthcare or agriculture applications requires standardized production protocols

DASbox® Mini Bioreactor System
> Process development, media development, strain characterization, clone screening
> Parallel setup of up to 24 bioreactors
> Excellent scalability and reproducibility
> Small working volumes save on the amount of cell material, media, and
> supplements required

Workflows for evaluating microbial communities

High-level community profiling → **Marker gene studies (16S rRNA. ITS or 18S rRNA)**

Functional profiling → **Metagenomics**

Real-time functional profiling → **Metatranscriptomics**

Sample storage
It is crucial for microbiome analysis to avoid variation during sample collection and shipping, and to allow for storage at -80 °C.

Challenges
× Adequate storage space for up to 10.000+ samples
× Temperature stability

Solutions
✓ High capacity per footprint
✓ Temperature stability and fast recovery times
✓ eLABinventory for sample management

Sample extraction and library preparation

Challenges
× Sample integrity and complexity
× Inconsistent sample quality and yields
× Error-prone long manual procedures
× Limited throughput

Solutions
✓ Liquid handlers help to speed along a standardized workflow, process more samples faster, reduce human error,
✓ and prepare samples more cleanly and accurately
✓ Automated sample extraction protocols on the ep*Motion*:
> MoBio, QIAGen and M&N extraction protocols
✓ Established library preperation methods on the ep*Motion*:

> Illumina TruSeq® Nano DNA
> Illumina TruSeq DNA PCR-free
> Illumina Nextera® XT DNA
> Illumina Nextera DNA Flex
> KAPA BioSystems® KAPA HyperPrep DNA
> KAPA BioSystems KAPA HyperPlus
> KAPA BioSystems KAPA HTP
> KAPA BioSystems KAPA LTP
> New England Biolabs® NEBNext® Ultra DNA
> New England Biolabs NEBNext Ultra II DNA Illumina TruSeq Stranded Total RNA
> KAPA BioSystems KAPA HyperPrep RNA
> KAPA BioSystems KAPA HyperPrep RNA with RiboErase
> Illumina® 16S Protocol (Customer Qualified)

Live microbial biologics + Culture of component microbes → **Bioprocessing**

Challenges
× Process parameters and medium compositions

Solutions
✓ Bioprocess control systems allow close process monitoring and control
✓ Small working volumes and parallel design save resources and time
✓ Bioprocess control software supports design of experiments and advanced statistical data analysis

References:
[1] Knight, R. et al. Best practices for analyzing microbiomes. Knight, R. et al. *Nature Microbiology Review* 2018; 16:410-422.
[2] Thompson, L. et al. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 2017; 551:457-453.
[3] Allen-Vercoe, E. Bringing the gut microbiota into focus through microbial culture: recent progress and future perspective. *Curr Opinion in Microbiology* 2013; 16:5.

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