Genomics Workflow Tools for Microbiome Research

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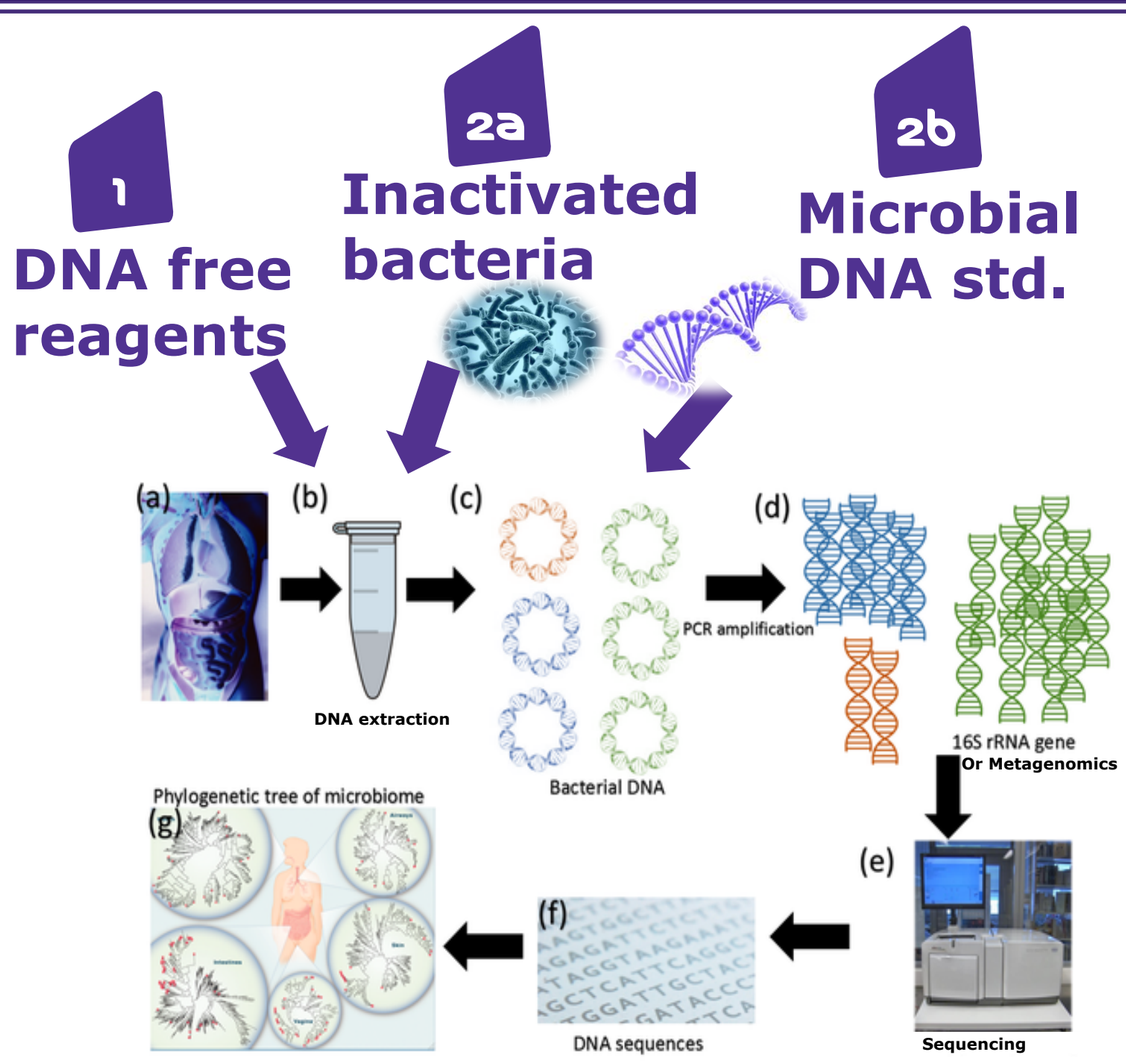
Abstract

Microbiome research is a rapidly expanding field. It is currently dominated by gut microbiome and its application to health and disease states, while other niches in human body and plants are also attracting a lot of attention. Progression in Next Generation Sequencing (NGS) technology now allows identification and sequencing of microbes in relatively low quantities and in complex samples. Therefore, NGS is the method of choice for population identification in microbiome samples, as most of them include large variety of microbes and in some cases also host cells. Still, biases and errors are common in this workflow and can be introduced in different steps along this process. To avoid bias in microbiome analysis, standardization must take center stage. It is an important part of the future of microbiome and metagenomic research to generate accurate and valid data. In addition, reagents used along this process must be free of any microbial contaminant to avoid introduction of exogenous error to the samples especially these with relatively low microbial counts. We here present standardization tools such as microbial DNA and inactivated bacteria microbiome standards that are suitable for PCR, sequencing and NGS as well as various lytic enzymes which are microbial DNA free. Usage of microbial DNA free lytic enzymes, which are necessary for sample processing, with individual microbial standards provide not only a solution to minimize contamination in sample processing but also specific customized control which can provide reproducibility and allowing reliable comparison of results lab to lab.

Many reagents include leftovers of microbial DNA which usually do not generate issues in downstream procedures. However, these may generate biases and errors when dealing with microbiome samples with low bacterial biomass. To address this challenge we are introducing a suite of microbial DNA free reagents such as lytic enzymes as described below.



Lack of standardization can lead to biases and errors in common processes during sample preparation and analysis such as DNA extraction, sample amplification, sequencing and bioinformatics analyses. Whole cell inactivated bacteria and microbial genomic DNA standards can be used in order to enhance the quality and reproducibility of the results.

Microbial DNA Free Lytic Enzymes

1a **Lysostaphin** (**SAE0091**) free of DNA contaminants, suitable for Microbiome research, lyophilized powder, ≥500 units/mg protein.

1b **Mutanolysin** (**SAE0092**) free of DNA contaminants, suitable for microbiome research, lyophilized powder, ≥4000 units/mg protein.

Gram Positive
Capable of cleaving the crosslinking pentaglycine bridges found in the cell wall peptidoglycan of certain **Staphylococci**.

Gram Positive
For bacteria such as **Listeria**, **Lactobacillus** and **Lactococcus**.

Yeast
Combines endoglycanase and protease activities that act together in **yeast** cell lysis.

Fungi
Chitinase is an extracellular enzyme complex that degrades chitin and hydrolyses the **fungi** cells wall.

1c **Lyticase** (**SAE0098**) free of DNA contaminants, suitable for microbiome research, ≥2000 units/mg protein, lyophilized powder.

1d **Chitinase** (**coming soon**) - free of DNA contaminants, suitable for Microbiome research.

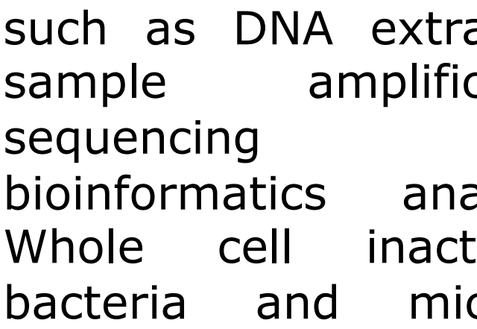
Validation of DNA free lytic enzymes by 16S rRNA PCR

1 - 4µgP from lyticase
2 –4µgP from lyticase
3 – microbial DNA+ 4µgP from lyticase
4 – microbial DNA+ 4µgP from lyticase
5 – Positive control
6 – Negative control
7 – 1Kb ladder

Microbial DNA and inactivated bacteria microbiome standards

Microbial inactivated bacteria

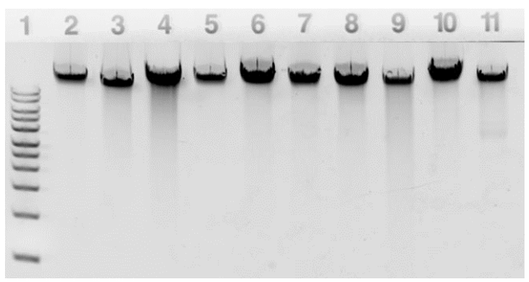
2a Whole Cell inactivated bacteria are provided at >10^8 bacteria/vial as liquid or as lyophilized powder. Suitable for DNA extraction, PCR, sequencing and next generation sequencing. We guarantee ≥95% identity of the specified bacteria (by NGS). These standards can be used as stand-alone or spike-in controls.



Inactivated *Porphyromonas gingivalis* **(MDB0009)**
1: 1kb DNA marker
2: Extracted genomic DNA,
3: 16S amplified product

Single microbial genomic DNA standards

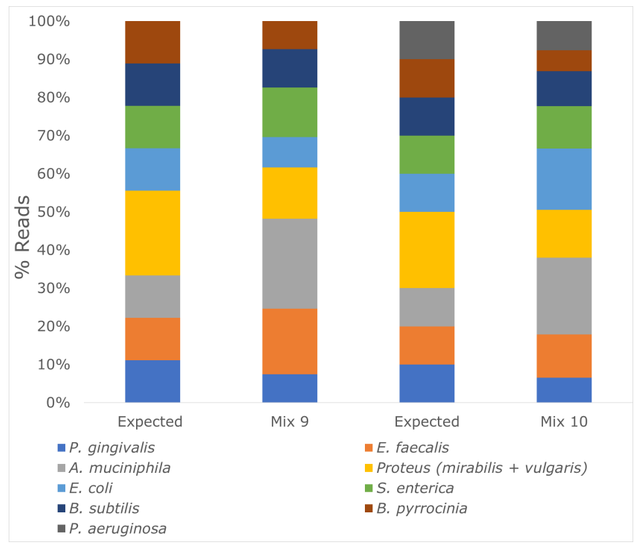
2b



Genomic DNA standards from various bacteria –
1*: 1kb DNA marker*
*2: A.muciniphila***(MBD0001)**
3: *B.subtilis* **(MBD0018)**,
4: *B.pyrrocinia* **(MBD0019)**,
5: *E.coli* **(MBD0013)**,
6: *E.faecalis* **(MBD0012)**,
7: *P.Aeruginosa* **(MBD0014)**,
8: *P.gingivalis* **(MBD0004)**,
9: *P.mirabilis* **(MBD0002)**,
10: *P.vulgaris* **(MBD0003)**,
11: *S.enterica* **(MBD0005)**

Microbial community DNA mix

2c



Microbial Community DNA mixtures suitable for PCR, sequencing and NGS are currently being developed and validated in two different labs. Bacteria species represent gram-positive, gram-negative, variable GC content and variable 16S copy number.