Microbiota Vault

Feasibility Study

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1. Executive summary

This study was initiated by a group of scientists with the goal of evaluating the feasibility of establishing the Microbiota Vault, an institution for the safe long-term preservation of microbial diversity. The independent professionals commissioned to perform the study assessed the concept and explored viable approaches for its implementation in a multi-step process. The study was performed in close interaction with a broad panel of internationally renowned experts.

The rationale for the Microbiota Vault initiative rests on the premises that a) microbial diversity is of great importance for human well-being and health and that b) microbial diversity is globally threatened by westernization, urbanization, and environmental change proceeding at an unprecedented pace, resulting in risks and lost opportunities. The initiative therefore strives to support the collection of this diversity while still possible and to establish a safe repository for long-term preservation, in analogy to the Svalbard Global Seed Vault. With its focus on long-term preservation and international collaboration, the initiative differs from all other microbiota collection efforts oriented towards research and characterization.

The study concludes that the rationale described above is sound and that the Microbiota Vault initiative is of high significance and potential. In order to fulfil its role, the Microbiota Vault as a repository will need to interact with existing collections and ongoing collection efforts globally and to build bridges between developing countries (often the hosts of such diversity) and developed countries (often the hosts of technical and scientific expertise and research activities on microbial diversity and functions). Unlike the Svalbard Global Seed Vault and seed banks, the Microbiota Vault cannot benefit from a preexisting international treaty (International Treaty on Plant Genetic Resources for Food and Agriculture), requiring it to engage in active approaches to address stakeholders and stimulate policy development (see “International Microbiota Vault Network” below). Further, it will need to interact with research on long-term preservation of microbial specimens and to adopt developing standards of specimen annotation. Finally, as an initiative of international scope, it will need to embed itself on an international political level, providing a strong value proposition for both developing and developed countries, in a spirit of benefit-sharing.

Having researched viable approaches to set up a Microbiota Vault from technical, legal, organizational, and ethical perspectives, the study recommends that the Microbiota Vault initiative adopt a governance design that is non-profit and inclusive, allowing participation of the relevant stakeholders and building sustainability parameters over time. Such a Microbiota Vault organization will be responsible for erecting and maintaining the physical Vault infrastructure.

In order to bridge to local research ecosystems and collections, to develop research partnerships, and to drive the initiative on the political level, the organization should adopt a network approach: an International Microbiota Vault Network should be established alongside the organization and utilized for stakeholder management and collaborative development of protocols and international standards.

Regarding the physical infrastructure, the study identifies two options: a) using cryopreservation (better established, but requiring active cooling) at a stable, neutral, well-connected site, ideally associated with an existing biobank; b) using lyophilization (less well established, not requiring active cooling) at a site in an arctic climate, similar to the Svalbard Global Seed Vault. The study recommends using option b) lyophilization in a backup capacity to option a) cryopreservation. Based on political and legal considerations and prior development efforts by the Microbiota Vault initiative, siting options in Switzerland and Norway were studied in detail. Both countries are well-suited for a cryopreservation facility, while a backup facility for lyophilized specimens could be sited on Svalbard, Norway, alongside or potentially as part of the Svalbard Global Seed Vault.

Regarding the modus operandi of the Microbiota Vault, the study recommends that the Vault store specimens *on behalf* of the associated local collections, with any future use of the specimens occurring via these local collections, thus reducing legal complexity on the part of the Vault. However, the Vault should not act as a passive repository, but actively shape the interaction with local collections on several levels: it should a) collaborate with the local collections and research partners in establishing common protocols and standards and establish a common legal-ethical framework. It should b) create added value by establishing a framework for annotation and metagenomic characterization, both of which should be openly available on a dedicated digital platform. And it should c) actively foster local collection efforts aimed at capturing still-existing microbial diversity, e.g., by supporting collection efforts in developing countries.

Recognizing that the establishment of a Microbiota Vault at a global international scale and with the scope described above will be a multiphase and expanding process, the study recommends the following course of action:

1. Establishment of an office to build the organization and international network as well as to drive the development at the political level.
2. Establishment of a proof-of-principle pilot project that demonstrates the concept, including a) installation of a pilot biobanking infrastructure in a site such as Norway or Switzerland, b) initiating a pilot collaboration project for sample collection with a local working collection in a developing country, c) performing annotation and metagenomic characterization of such samples, and d) shipping of such samples from the local working collection to the biobanking infrastructure. Concretizing the Microbiota Vault concept in such a fashion will be instrumental in addressing the political level and scaling.

To limit complexity in the first phase, the study recommends initially focusing on human-body-associated microbiota samples only, considering current research priorities and legal factors. Conceptually, it should however not restrict itself to human-body-associated microbiota for the future.

Taking into account the establishment of an organization and management office and the execution of a pilot project as described above with a duration of two years, including the establishment of own infrastructure and including metagenomic analysis, cost is estimated in the range of USD 816'000 - 1'210'000. Depending on infrastructure choices, lower cost options are conceivable.

The study was financed by multiple non-profit organizations and universities active in the microbiome space, including Seerave Foundation, Gebert Rüf Foundation, Rutgers University, Calouste Gulbenkian Foundation, Kiel University / Kiel Life Science, UC San Diego Center for Microbiome Innovation / UC San Diego School of Medicine, Canadian Institute for Advanced Research (CIFAR), and Bengt E. Gustafsson symposium foundation, affiliated with Karolinska Institutet.

The study was conducted by the Swiss companies advocacy and EvalueScience.

This study was finalized in the midst of the COVID-19 pandemic sweeping across the world. It is to be hoped that this unprecedented crisis will call attention to the value of precautionary approaches, as they are core to the Microbiota Vault initiative – not least given the importance of biodiversity for resilience against disease.

2. Initial situation and assignment

2.1. Relevance of microbiota and microbial diversity

In recent years, our awareness of the fundamental role microbiota play in human life and well-being has greatly expanded[1, 2]. Research into human-associated microbiota and their collective genomes, the microbiome, has accelerated (the same applies for animal and plant microbiota). It has become clear that microbiota are an integral part of human physiology: not only are microbiota essential for digestion; they also interact with the immune system, metabolism and other physiological systems[3]. There is growing evidence that microbiota play a role in chronic diseases such as inflammatory bowel diseases[4], asthma[5, 6], cancer[7], and type 2 diabetes[8].

As a collective entity, human-associated microbiota comprise a diverse set of bacteria, viruses, fungi, and protozoa, whose composition differs between individuals, populations, cultures, and lifestyles[9]. The diversity and composition of the microbiome is in itself an object of intense study[10, 11]. For example, there is evidence that nutritional lifestyles associated with metabolic syndrome are associated with changes in the diversity and composition of the gut microbiome[9, 12]. There is ongoing research regarding the protective effects of a diverse microbiome against pathogens[9, 13]. Not least, there is serious concern that widespread use of antimicrobials and antibiotics leads to changes in the composition of human microbiota, with detrimental effects on human health[14, 15], e.g., reducing resilience against *Clostridium difficile* infection[16].

Currently, the global diversity of human-associated microbiota is threatened by the global alignment ("westernization") of lifestyles in the context of urbanization and the shrinking of indigenous cultures, in which a much higher microbial diversity has been observed[10, 17].

While the scientific discovery of causal relationships between individual microbes or microbial communities and human health is still in its infancy, means to protect and preserve microbial diversity *now* may become critical to conserve long-term human health in the future.

2.2. Feasibility study for the Microbiota Vault initiative

Within the Microbiota Vault initiative, a pioneer team of international experts has come together with the aim of safeguarding microbial diversity by supporting collection efforts and creating an institution for safe preservation, the Microbiota Vault. The initiative takes inspiration from the Svalbard Global Seed Vault, which safeguards the global diversity of food crop seeds.

The initiative is supported by several nonprofit foundations and academic institutions, which commissioned the present feasibility study in order to assess and concretize the concept and to work out scenarios for its implementation.

The following institutions supported the feasibility study:

	Seerave Foundation
	Gebert Rüf Foundation
	Rutgers University
	Calouste Gulbenkian Foundation
	Kiel University / Kiel Life Science
	The Microsetta Initiative / UC San Diego School of Medicine
	Canadian Institute for Advanced Research (CIFAR)
	Bengt E. Gustafsson symposium foundation, affiliated with Karolinska Institutet

3. Objectives of the feasibility study and course of action

3.1. Objectives

The aim of this feasibility study is to determine whether the Microbiota Vault idea can be implemented and if so, to identify viable approaches. This entails assessing and refining the concept, working out scenarios for implementation, checking their feasibility, and appraising the pros and cons. The results of the study are intended to offer a framework for further decision-making regarding the shaping and building of the Microbiota Vault initiative.

3.2. Course of action

The feasibility study was conducted in close collaboration with a project management group from the Microbiota Vault initiative consisting of

- Prof. Maria Gloria Dominguez-Bello, Center for Human Evolutionary Studies, Rutgers University
- Prof. Martin J. Blaser, Center for Advanced Biotechnology and Medicine, Rutgers University
- Dr. Manuel Fankhauser, Chief Scientific Officer, Seerave Foundation

The study was performed in several phases (see Figure 1). After fixing project outlines and selecting experts for interviews during a kick-off workshop, research and concept development was performed. The results of this phase were synthesized and presented at a second workshop. In the following second research phase, certain aspects were researched in more detail, including a closer mapping out of pilot setups for the Vault. Finally, the present report was generated.

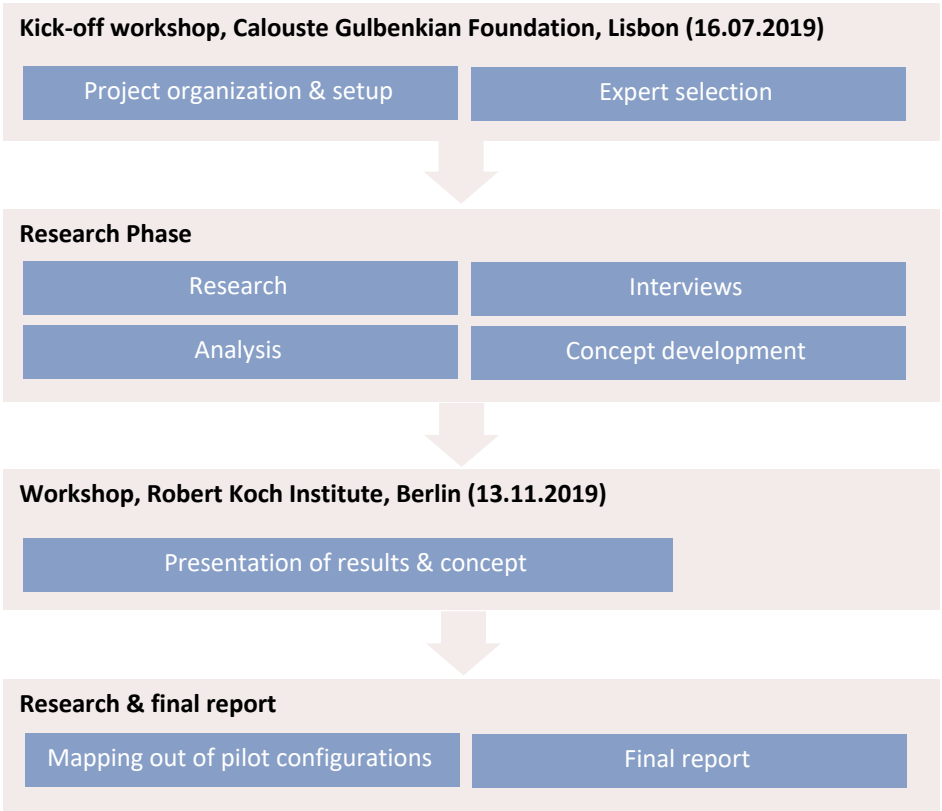

Figure 1: Project Path

During the study, the following experts were interviewed:

Interviews during research phase (semi-standardized interviews)	
Hannes Dempewolf	Senior Scientist and Head of Global Initiatives at the Crop Trust
Joël Doré	Director of Research, INRA, Paris
Cary Fowler	Former executive director of the Crop Trust, currently serving as a Senior Advisor
Keiji Fukuda	Director and Clinical Professor, Division of Community Medicine and Public Health Practice, School of Public Health of The University of Hong Kong
Sascha Ismail	Scientific employee, Forum Biodiversität Schweiz, Swiss Academy of Sciences (scnat)
Rob Knight	Professor of Pediatrics and Computer Science & Engineering, University of California, San Diego
Marc LaForce	Professor, NYU Langone School of Medicine
Daniele Manzella	Treaty Technical Officer, International Treaty on Plant Genetic Resources for Food and Agriculture, Food and Agriculture Organization (FAO) of the United Nations
Tore Midtvedt	Emeritus, Karolinska Institute, Department of Microbiology, Tumor and Cell Biology
Richard Roberts	Chief Scientific Officer at New England Biolabs
Manuel Schmidt	Deputy Director, Instituto Gulbenkian de Ciência
Erica Sonnenburg	Senior Research Scientist, Sonnenburg Lab, Stanford School of Medicine
Justin Sonnenburg	Associate Professor of Microbiology and Immunology, Stanford School of Medicine
Shinichi Sunagawa	Assistant Professor of Microbiome Research, Institute of Microbiology, ETH Zurich
Herbert Zech	Professor of Civil, Technology and IT Law, Humboldt-Universität zu Berlin, Director Weizenbaum Institute for a Networked Society
Additional interviews regarding the setup of pilot configurations	
Sabine Bavamian	CSO, Swiss Biobanking Platform
Dominique A. Caugant	Chief Scientist, Norwegian Institute of Public Health
Gottfried Dasen	CEO, Culture Collection of Switzerland / ZHAW School of Life Sciences and Facility Management
Adrian Egli	Professor, Clinical Microbiology, University of Basel
Carlo Largiadèr	Professor, University Institute of Clinical Chemistry, University of Bern / Inselspital / Biobank Bern
Ørjan Olsvik	Professor, UiT The Arctic University of Norway
Michael Scharl	Professor, Department of Gastroenterology and Hepatology, University Hospital Zurich / Translational Microbiome Research Center
Christoph Scheidegger	Professor, Swiss Federal Institute for Forest, Snow and Landscape Research WSL / Head Platform Biology, Swiss Academy of Sciences (scnat)

Listed below are the core questions that have been analyzed during this feasibility study:

- How can the Vault idea be concretized into a workable and sustainable framework?
- What is the scope of the Vault?
- How does the Vault relate to microbial and health research?
- What are the relevant technical, legal, and ethical issues?
- How is the Vault embedded in the international political landscape?
- Who are the relevant stakeholders?

4. Conceptualizing the Microbiota Vault

4.1. Rationale for a Microbiota Vault

The Microbiota Vault initiative sets out to preserve the diversity of human-associated microbiota by constructing an institution for the safe storage and preservation of microbiota samples and collections. Such samples and collections are to be made available for future resuscitation, culturing, and research based on clearly defined rules such as established by a dedicated international treaty.

The rationale for such a "Noah's Ark" of microbiota rests on two intertwined premises:

Firstly, human-associated microbiota play a significant role in the biology of humans and in human health. They have co-evolved with their human hosts, many of them forming commensal and symbiotic relationships. Microbiome research constitutes a young and rapidly expanding field of research, with the majority of work being done in the field of gut microbiota, but also going beyond. Microbial communities are highly diverse, both between individuals and populations. Understanding this diversity is key to understanding the role of microbiota. There is growing evidence that changing lifestyles in the context of westernization and urbanization go hand in hand with changes in the composition of gut microbiota[18]. The excessive use of antibiotics - and, as recently shown, also of non-antibiotic drugs[19] - has profound effects on the composition of the microbiome.

Secondly, the very same global developments of westernization and urbanization have an irreversible impact on the global diversity of human-associated microbiomes. This process occurs at a point in time when research has just started to understand the relevance of this diversity. Possibly, ongoing impoverishment of human-associated microbiomes will increase risks (e.g., prevalence of chronic diseases or resilience against pathogens[20]) and decrease opportunities (e.g., probiotics, microbiota as sources for drugs and therapies[17]). Environmental change caused by humans proceeds at an unprecedented pace, likely also affecting animal and plant microbiota[21].

At present, only a fraction of microbial diversity is known. According to Lagier et al. (2018), of 2'671 human-body-associated species of microbiota known at that time, 1'057 species were only recently cultured from stool samples using new methods of comprehensive culturing ("culturomics"). The Microsetta Initiative has observed over 1'000'000 16S V4 sequencing fragments (sub-Operational Taxonomic Units (sOTUs), or unique sequence variants) from human samples (primarily fecal, skin, oral)[23]. Metagenomic analyses had previously shown that ~80% of the bacteria inhabiting the human body are unknown, prompting the metaphor of "microbial dark matter". Such unknown diversity also extends to archaea, microbial eukaryotes, and viruses.

Taken together, this means that there is a danger of irrevocably losing valuable information and opportunity, at a time when science has just started understanding the health relevance and potential of our microbial environment and the microbiome.

Hence the need for a global collection of such microbiota and for their safe storage and preservation. The Microbiota Vault initiative strives to enable such safe storage (the Microbiota Vault itself), but it also considers the question of fostering regional collection efforts and partnering with other regional and local collections that are open for research and that can backup specimens in the Microbiota Vault. While many initiatives and collections exist with an aim to study the microbiome, the Microbiota Vault initiative is unique in its focus on long-term preservation of microbiota, and its promotion and support of regional collections, particularly in locations with high microbial diversity. In analogy to the Svalbard Global Seed Vault, the Microbiota Vault is intended to act as a backup for local efforts (in the Seed Vault's case the local seed banks) and hence will need to interact with local partners on an international scale. In the context of their

relationship with the Vault, such local collection efforts are called "local working collections" in this document.

4.2. Initial findings and requirements placed on the Vault

In the course of the interviews performed during this study, it became evident that the Microbiota Vault is widely seen as an innovative initiative with high significance and potential. Thanks to the input of a diverse set of experts and the research performed during the feasibility study, the requirements for such a project became more apparent.

On a high level, it became clear that similar to the Svalbard Global Seed Vault, the Microbiota Vault cannot act on its own. It will need to interact with existing collections and ongoing collection efforts globally, and will need to build bridges between developing countries (often the hosts of such diversity) and developed countries (often the hosts of technical and scientific expertise and research activities on microbial diversity and functions). Unlike the Global Seed Vault, the Microbiota Vault cannot bank on preexisting international treaties, as is the case with seed banks.

Furthermore, if the Microbiota Vault aims to preserve the worldwide diversity of microbiota over a long period of time, it will have to be unique in a sense that, unlike other collection efforts, the first goal will not be the analysis of specimens, but preservation in a form that will allow future resuscitation and culturing.

On a more detailed level, we conclude that the Microbiota Vault will have to meet the following requirements:

The Vault will need to

- be situated and set up so as to safeguard microbial samples over long periods of time (as long as the current state-of-the-art methods allow);
- be connected to existing collections that perform research, and to local collection efforts (in order to capture and annotate the diversity of microbiota);
- interact globally;
- present an attractive offer to developing countries which harbor microbial diversity;
- present an attractive offer to developed countries which support microbiome research and have high interest in the health-related roles of microbiota;
- be set up and act in a way that enables benefit-sharing;
- be compliant with the relevant legal and ethical frameworks (medical, biodiversity, access to genetic resources);
- be set up in a manner that enables small beginnings and subsequent growth;
- be set up in a way that allows its continued operation in a sustainable way.

Samples need to

- be stored in a way that optimizes the chances for resuscitation and culturing;
- be annotated with metadata that receives influx of information on the characterization of the stored samples, that is transparent and open access, optimizing use of information and use of the specimens in the local/regional collections.

4.3. Comparable projects and initiatives

Appendix 8.3 gives an overview of the landscape of microbiota collections, organizations and structures relevant for the preservation of biodiversity, and of seed bank and seed vault organizations.

Microbiota collection occurs chiefly in academic research initiatives, with the goal of characterizing microbiota (genomic, metabolomic, transcriptomic, interactions with host). The vast majority of these initiatives are situated in the United States, Europe, and China.

With its proposed close relationship to research (see below) and its primary goal to preserve, the Microbiota Vault initiative is related to such collection efforts, but it also shares traits with seed banks and vaults for crops and plants, particularly with the Svalbard Global Seed Vault.

Svalbard Global Seed Vault

Established in 2008, the Svalbard Global Seed Vault operates under the Multilateral System of the International Treaty on Plant Genetic Resources for Food and Agriculture, in a tripartite agreement between the Government of Norway, the Nordic Genetic Resources Center (NordGen), and the Global Crop Diversity Trust (Crop Trust). It stores approximately 1 million seed samples on behalf of seed banks participating in the Multilateral System. Its arctic and remote location in a former coal mine was chosen to allow storage of samples in optimal conditions (-18 °C) that can be maintained without human intervention. Samples are preserved as backups on behalf of the seed banks, ensuring survival of the seeds in case they are lost locally. The Svalbard Global Seed Vault operates a "black box" system, with donor banks owning and controlling access to the seeds they have deposited.

The Seed Vault is owned and administered by the Ministry of Agriculture and Food of Norway, in collaboration with the Global Crop Diversity Trust, which supports operations and provides funding for the preparation and shipment of seeds from developing countries. The facility is operated by Nordic Gene Bank (NordGen) which also maintains a public database of samples stored in the Vault. An International Advisory Council oversees management and operations.

5. Building the Microbiota Vault

5.1. General considerations

This chapter lists three overarching considerations whose importance became evident during interviews and research. They are reinforced by the more detailed analysis described in the following chapters.

1. Safeguarding samples over time

The Microbiota Vault initiative proposes to safeguard microbial diversity by providing a safe place for long term storage on behalf of local and regional collections around the world. In the case of the Svalbard Global Seed Vault, storage at a remote, arctic location on the island of Svalbard was chosen, allowing survival of the storage collection in the absence of human intervention. This constitutes a possibility for a Microbiota Vault using a specific storage mode such as lyophilization (see below). Otherwise, specimens containing bacteria need ultralow temperatures to be preserved, in a way similar to established biobanks. Such an operational mode would call for a well-accessible, actively maintained, more central, politically stable location.

Implementing independent safeguarding methodologies and locations in parallel would build redundancy in terms of location, and increase the chances of resuscitation. To assess the different options, several factors have to be considered. Important among them are overall risk over time, cost, storability of samples, connection to research.

2. The Vault and research

While the Microbiota Vault initiative primarily focusses on preservation, it is very closely linked to research on several levels. Firstly, it needs to interact with efforts to collect microbial diversity. Secondly, long-term safe storage of microbial samples is a research question in itself. Thirdly, and perhaps most importantly, the initiative derives an important part of its appeal from its potential to catalyze research into the diversity and health relevance of microbiota. To bootstrap and scale the initiative, it is therefore critical to arrive at a convincing model for the interaction of the Vault and research.

3. International scope

With its goal to safeguard the global diversity of microbiota, the initiative is of international scope. It can only be scaled if it succeeds to embed itself on an international political level, transcending the realm of academia and research. This also entails that a strong value proposition must be developed, not only for developed countries with a strong interest in microbiota-related health concerns, but also for developing countries harboring an important part of the diversity, in a spirit of benefit-sharing.

5.2. Project analysis

5.2.1. Technical analysis

Sample collection and preservation

Samples to be stored in the Microbiota Vault form two categories:

- Complex samples, i.e., specimens containing complex mixtures of microbiota
- Axenic samples, i.e., single strain cultures of microbial organisms

We foresee that at least initially, collections in the Vault will, for the following reasons, mainly consist of complex samples: if the Vault is to capture the diversity of (presently unknown) microbiota, the prime source

material will consist of such specimens. These Vault specimens will be backups of specimens in local/regional collections that are amenable to research. In order to capture the full diversity present in a complex sample, high-throughput methods of culturing would have to be employed to derive axenic cultures. While the development of such methods has made great strides ("culturomics"), they are very resource-intensive, and it is not assured that these methods allow exhaustive capture. Concurrently, storage space requirements would be very high if individual strains were to require separate storage. In terms of sample type, one has to consider that the main focus of current microbiome research lies in human gut microbiota. While the Vault will not restrict itself to specific types of samples and should be open for the preservation of the microbiomes important for human health, it is likely that the main use in the near future will be related primarily to fecal samples.

Regarding the long-term storage of samples, two primary modes are possible:

- Cryopreservation (storage at very low temperatures, typically at -80 °C in refrigerators or at <-130 °C using liquid nitrogen)
- Lyophilization (freeze drying)

Currently, knowledge on long-term preservation of microorganisms is still limited[24, 25] and only a few methodologies for cryopreservation of non-axenic cultures have been described[26]. Yet, cryopreservation is better established than lyophilization and is currently considered the standard method for fecal samples. As Smirnova et al. (2019) summarize:

> To date, cryopreservation at −80 °C with the addition of glycerol as a cryoprotective additive is the main way to preserve the microbiota in fecal samples.
> Current cryopreservation methods are well established for pure cultures, but there are no standardized protocols for preserving ecosystems at the complex microbial community level. The cryobiological studies related to the conservation of mixed and enriched cultures, natural microbial communities and fecal transplants are at the early stages of development.
> The vast majority of works on cryopreservation of the human intestinal microbiota have been carried out using fast freezing techniques with a limited storage time that ranges from a week to 12 months at −80 °C. Only in individual studies storage at −196 °C is recommended to prolong the cryopreservation time.

For true long-term storage, temperatures below the glass transition temperature of water (-130 °C) should be used, typically requiring storage systems depending on liquid nitrogen, which calls for regular replenishment.

Lyophilization is less well researched, but considered to be associated with a lower potential for successful resuscitation after storage. It has the advantage that lyophilized samples can be stored at higher temperatures (at or below 4 °C).

While it is still early days regarding our knowledge about long-term storage and subsequent resuscitation and cultivation of complex samples, it is important to recognize that research into these problems is currently accelerating. Until the advent of culturomics, collection and storage protocols were mostly employed for the characterization of complex samples (nucleic acid analyses, metagenomics), and not with a view to preservation, resuscitation, and cultivation. Next to culturomics, the high interest in health applications of gut microbiota, particularly regarding fecal microbial transplants (FMTs), has accelerated research with respect to optimal protocols. A lot of research and development regarding optimal and standardized storage is performed with the goal of developing FMTs into commercial therapeutic products. In this context, new methods assisting the collection and preservation of samples (collection devices, preservatives) are currently being developed, e.g., patents on lyophilization techniques[28].

With regard to fecal samples, assuring sample integrity after collection is critical if the viability of the microbiota is a concern, as in the case of the Microbiota Vault. Oxygen-sensitive species form an important

part of gut microbiota. They require immediate protection after collection. Immediate deep freezing of samples is the best-established method. Different preservatives are currently being used, depending on the storage method and collection goals (e.g., a specific preservative might be compatible with preservation/resuscitation, but might be incompatible with specific types of analytics).

In sum, the current state of knowledge about sample collection and preservation means the following for the Microbiota Vault:

- Collection and storage protocols will have to be co-developed and adapted over time, and ideally standardized for the use of local/regional collections that integrate the system. The establishment of such protocols constitutes a research endeavor in itself and necessitates a close interaction with research. Methods for collection and preservation are currently in development which might change the points below.
- Immediate deep-freezing of fecal samples as early as possible after collection currently yields the best prospects for later resuscitation.
- Cryopreservation is currently the storage method with the best prospects for preservation and resuscitation.
- Lyophilization is attractive because it enables storage at temperatures as they occur at arctic locations, without the need for active cooling. It is however less well established and may lower viability of multiple strains.
- For optimal compatibility with subsequent cryopreservation and lyophilization, the choice of preservatives/protectives has to be carefully evaluated.

Sample annotation and characterization

Next to the establishment of protocols for sample collection and storage, setting up an annotation system for the contents of the Vault and for the sample characterization updates performed in research centers will be important for optimal dissemination of the information, for the fostering of further research and for the purpose of enabling future use. A minimal dataset specifying the provenience and nature of samples to be stored in the Vault will need to be developed. It should be designed to be compatible with existing public repositories and in adherence with best practice. Definition of such a dataset should occur in collaboration with the research community and could make use of established standards from preexisting initiatives.

While the Vault in itself is not a research endeavor, it could produce a benefit and simultaneously heighten its attractiveness by combining sample storage with an offer for deeper characterization of the samples via metagenomics or possibly further -omics approaches. Such a characterization could take place with the associated local working collections supplying samples and via partnerships with existing characterization efforts.

Both the annotation and characterization of samples would need to be accessible in an open and transparent manner (e.g., via a dedicated digital platform), even if the access to physical samples were to be restricted to the donating local working collection. This transparency would ensure equal opportunity in knowing the microbial diversity at any given point in time, and be key in facilitating global collaborations between researchers and local working collections.

5.2.2. Organizational analysis

Currently, the Microbiota Vault initiative is driven by an informally organized consortium of visionary individuals, many of them scientists of eminent standing. As a first step in concretizing the initiative, the 501(c)(3) nonprofit public charity "Microbiota Vault Inc." was founded. Building on this, organizational forms

and forms of governance which are suited for launching the Vault in a pilot configuration and scaling the initiative on the international political level will need to be developed. The following aspects are important:

1. As a future organization of international importance, a Microbiota Vault organization will need to develop a governance design that is inclusive, foresighted, and that can build sustainability parameters over time. Such a design must allow credible participation of the stakeholders (research community via the network, local working collections, policy representatives, beneficiaries) so as to suitably represent their interests.
2. The organizational form and its governance must credibly reflect the principles of furthering the common good and benefit-sharing which are fundamental in creating trust and enabling a value proposition that works for the interests both in the developed and the developing world.
3. For a majority of experts, the connection from Vault to research is crucial. While the Vault's primary function will be to safeguard samples on behalf of local collections, a "pure" safe storage model is seen as at higher risk of not finding sufficient financing. The Microbiota Vault initiative's appeal is closely connected to the strong interest in the health relevance of microbiota.

The organization sustaining the Vault will therefore need to act as a bridge to research, and to foster an ecosystem that catalyzes sample collections locally at a global scale as well as research activities. We propose that a fundamental function of the Vault organization should be to create and orchestrate a network that builds bridges to local working collections, nurtures research partnerships, helps in stakeholder management, and is a forum for the development of protocols and standards. The concept of such an "International Microbiota Vault Network" is described in more detail in chapter 6.3.

An organizational setup that encompasses these aspects could look like this:

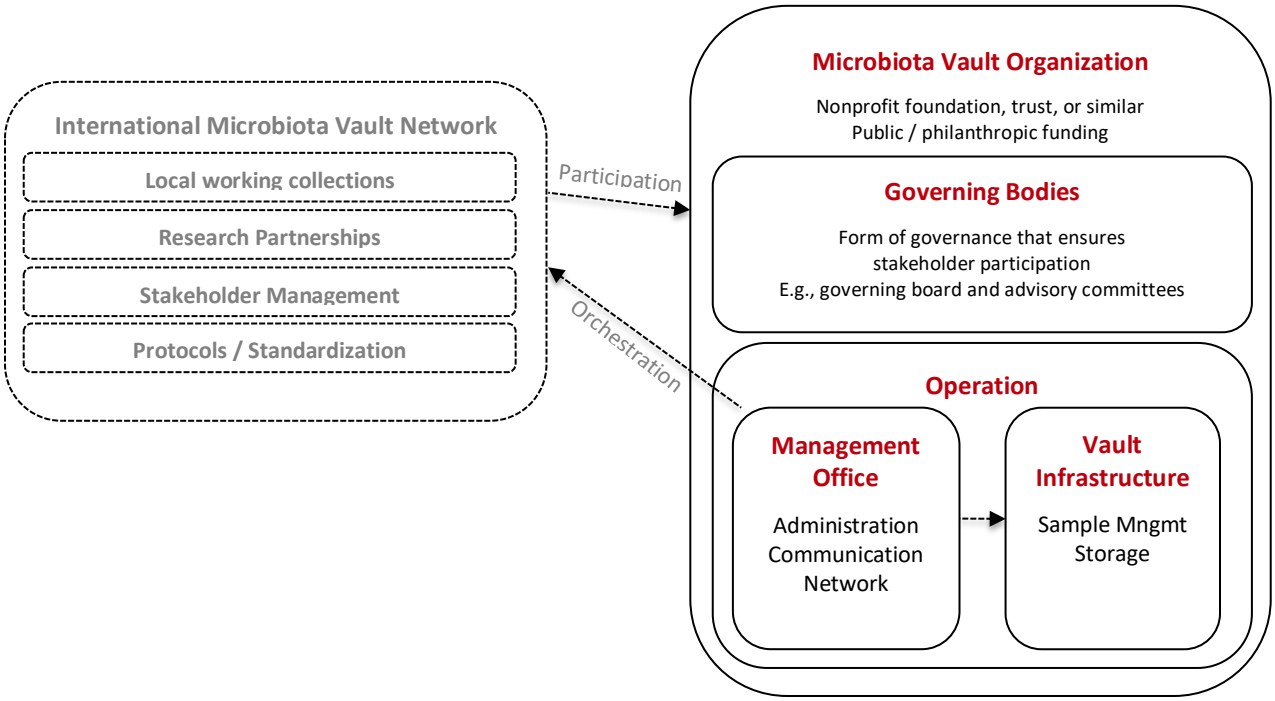

Figure 2: Organizational Form

5.2.3. Legal analysis

The Microbiota Vault is situated at the intersection of two systems of regulation:

- legislation addressing human research and personal data protection
- legislation concerning biological diversity and genetic resources, namely the Convention on Biological Diversity (CBD) and its supplementary agreement, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS)

The situation is relatively complex and different from the situation of the Svalbard Global Seed Vault, which operates under the International Treaty on Plant Genetic Resources for Food and Agriculture. In general, and for all systems of regulations, the law of the state where the Vault will be located prevails (be it human research law, data protection law, CBD). However, the laws of the donor states also need to be considered.

Human-body-associated microbiota (and the samples they occur in) are currently considered human samples. Human research legislation and personal data protection legislation therefore apply. For collection and storage of such samples, respective consents and permits are a necessity. The nature of such consents and permits depends on the question of research use of such samples.

In a model where the Microbiota Vault only stores samples *on behalf* of the associated local collections and any future use of the samples occurs via these local collections, the legal complexity on the part of the Vault is reduced. Characterization of samples in the Vault using metagenomics will have to be performed with the associated local collections, since doing this with Vault samples would likely constitute "use". This does not preclude that characterization of the samples by the local working collection (e.g., metagenomics) and subsequent submission of the associated data on a dedicated digital platform could be a precondition for sample deposition in the Vault. For the publication of metagenomic sequences, appropriate de-identification, filtering, and masking will need to be performed to exclude human sequences. In any case, local regulations regarding research with humans and data protection will apply to the local collections, e.g., authorization for indefinite storage of samples without personal identifiers by ethical committees or research boards.

In order to scale the Vault and its network activity (see below) as well as to assure legal compliance and engender trust in the model, standards and respective templates must be established.

Currently, there is an ongoing debate on whether human-body-associated microbiota fall under the Nagoya Protocol. At the moment, this question is being controversially discussed[29, 30], and no prediction is possible concerning if and when such an interpretation will be adopted by signatory states. For the Microbiota Vault, this question is of relevance, as it increases the complexity of the regulatory environment for one of its main use cases, i.e., human samples. Should environmental microbiota (not coming from human bodies) be included in the Vault, the situation is clear: these are subject to the Nagoya Protocol.

Signatory states of the Nagoya Protocol require contracts about access and benefit sharing. Importantly, this concerns both export and import states. The EU, Switzerland, and Norway are parties to the Nagoya Protocol (see rationale for specifically mentioning Switzerland and Norway in 5.2.5), as are most countries in Asia, Africa, and South America. The United States is not a party, but has certain provisions which resemble the Nagoya Protocol, i.e., proof of export permits are required. As is the case for legislation concerning human research, legal complexity is reduced if the Vault only stores samples on behalf of associated collections. In the context of positioning the Vault on an international level and in order to strengthen collection efforts in the developing world, it seems advisable to situate the Vault in a country that is part of the CBD/Nagoya system.

From an international policy perspective, it will be important to include stakeholders from all relevant domains and to adopt a flexible approach. This will also be necessary in the context of scaling the project and working towards anchoring the project on an international political and legal level. An international treaty

regulating and supporting the Vault, similar to the situation of the Svalbard Global Seed Vault, would be an optimal outcome. However, such a treaty could only be striven for in a second step, after the establishment of the Vault in the current legal frameworks, and the process would take years.

5.2.4. Ethical / cultural analysis

The Microbiota Vault initiative's primary goal to safeguard microbial diversity for the benefit of humankind is reflected in its planned organizational setup as a nonprofit organization that acts for the common good. When operationalizing the idea, the following aspects are relevant:

- The Vault will collaborate with local working collections (existing and new collections) which retain rights on the samples. While the responsibility remains with the depositors, the Vault needs to define criteria, standards, and a common ethical framework for collaboration. This is particularly important regarding standards for informed consent and, in the context of metagenomic characterization of samples, regarding standards for the protection of personal data.
- The Vault needs to document provenience of samples, including associated collection permits.
- As part of its activities with local working collections in the developing world, the Vault needs to foster a culture of participatory inclusion, respecting the interests of donor individuals and indigenous cultures. Collections should always involve participation of local teams, which hold the permits, deposit in the local collection, and obtain the needed permits to deposit a backup in the Vault.
- In the spirit of benefit-sharing (making sure that persons, institutions, and countries that contribute to the Vault, for example by means of samples, receive equitable benefits for their contributions), the Vault should protect the deposited samples, honor the right that the depositor holds on the samples, maintain public databases of samples and metadata (associated characterization), and foster a culture of open access to this information whenever possible.

5.2.5. Site analysis

A siting choice for the Microbiota Vault depends on several technological, political and organizational factors.

Considering **cryopreservation**, the most straightforward and sustainable option would be an association with an existing biobank in a well-connected, politically neutral, and stable location. Long-term access to cheap energy could be a plus. Association with an existing biobank would allow the Vault to profit from existing biobanking, biosafety and shipping infrastructure. Given the aims of the Vault, being headquartered in a politically neutral and stable location with well-developed links to international policy would suit the Vault in terms of political development and scaling of its mission. While the Vault may initially be hosted in existing infrastructure, it is conceivable that it would require dedicated infrastructure for scaling and long-term security. Both *de novo* or repurposed infrastructure, e.g., defunct army bunkers, are potential options. The initiators of the Microbiota Vault initiative have already identified potentially suitable and available army bunkers in Switzerland (managed by armasuisse). However, while these could be cost-effective options (given the selling prices are orders of magnitude lower than the cost of building such infrastructure), considerable legal and political efforts would be needed to convert them.

Of note, such a siting choice would differ from the concept of a "doomsday vault" that targets survival of the samples in the absence of human intervention when civilization breaks down. At least in the earlier stages of scaling up the Microbiota Vault project, this would nevertheless be a logical choice from a storage safety perspective, as cryopreservation is currently best supported by technical state-of-the-art, and storage protocols for long-term storage still have to be optimized.

Looking at **storage of lyophilized samples**, a "doomsday vault" configuration similar to the Svalbard Global Seed Vault would be possible. Samples could be stored in a remote, arctic location where they could survive

without active maintenance. Given that the viability prospects for lyophilized samples are less favorable compared to cryopreservation, such a storage mode would seem ideal as a backup for a more actively maintained cryogenic storage collection. Naturally, the Svalbard Global Seed Vault seems like a very favorable location for such storage, provided its specific purpose defined by the International Treaty on Plant Genetic Resources for Food and Agriculture would allow such an extension of scope.

Siting options for pilot configurations of the Microbiota Vault

The Microbiota Vault initiative has well-developed connections to both Norway (owner of the Svalbard Global Seed Vault) and to Switzerland. During the research for this feasibility study, we conducted interviews with stakeholders in both countries, mapping out potential pilot configurations for a Microbiota Vault. While these countries should not be considered exclusive choices, they might in combination satisfy the siting criteria described above.

For Switzerland, interviews were conducted with researchers connected to four biobanks being established in the vicinity of three university hospitals, as well as with experts from national research administration institutions. The contacted stakeholders expressed high preliminary interest in the Vault project and willingness to consider collaborations. Given broad stakeholder interest, a win-win situation might be created: local collaborators could profit from participation in a pilot Vault as an asset in the research landscape, and conversely the Vault could use such local stakeholder support to scale the project via the political level. Currently, considerable investments into microbiome research infrastructure are taking place in Switzerland (e.g., funding of a National Centre of Competence in Research (NCCR) dedicated to microbiome research, or establishment of a Translational Microbiome Research Center in Zurich). Furthermore, Switzerland might be a favorable location for pilot cryogenic storage due to its neutrality and connections to international policy (UN, WHO in Geneva).

For Norway, the interviews regarding possible pilot configurations were conducted with experts from UiT Arctic University of Norway and the Norwegian Institute of Public Health. Regarding siting a lyophilizate collection in the Svalbard Global Seed Vault, it is currently not clear if the political frameworks supporting the Seed Vault could encompass a Microbiota Vault lyophilizate collection. However, Svalbard would still seem an excellent choice for such storage. Via the UNIS University Centre in Svalbard, storage in coal mines similar to the one hosting the Svalbard Global Seed Vault could be established. Such a backup storage site could be combined with cryogenic storage in a better-connected location such as Tromsø or Oslo, as well as with cryogenic storage in Switzerland or another country.

5.2.6. Economic analysis

It is beyond the scope of this feasibility study to develop a full-fledged business plan encompassing a fully scaled Microbiota Vault organization, especially if the organization engages in capacity building with local working collections in the developing world. What can be said: such a mature stage will depend on positioning the initiative on the international level and securing country-level support.

We can however arrive at **cost estimates** (in USD) for the first steps needing to be taken to develop the initiative:

Organizational development and management office

We estimate costs for establishing the organization, running a management office, marketing and representing the initiative at USD 110'000 - 160'000 per year.

Personnel cost p.a. (60% FTE)	USD 60'000 - 85'000
Marketing, representation, conference organization, p.a.	USD 50'000 - 75'000
Total p.a.	USD 110'000 - 160'000

Sample storage

We have contacted the Cell Culture Collection of Switzerland (CCOS) for rough cost estimates for cryostorage of samples. The cost estimates are given with the assumption of storing 1'000 samples per year, over a period of 10 years.

The investment for setting up new cryo-facility infrastructure (tank connections, piping, loggers, ventilation, software, etc.) covering this capacity was estimated at CHF 276'000 (CHF being approximately at parity with USD [1 USD = 0.98 CHF as of February 2020]). See appendix 8.2.1 for the cost breakdown.

The cost for sample storage (including storage tanks) is strongly volume-dependent.

For 2 ml samples, average cost was estimated at CHF 27'000 per year, with a cost of CHF 93'000 for the first two years (1 tank for 10'000 samples). The total sample storage cost for 10 years amounts to CHF 267'000.

For 120 ml samples, average cost was estimated at CHF 104'000 per year, with a cost of CHF 190'000 for the first two years (2 tanks for 2'000 samples, 7 tanks for 10'000 samples). The total sample storage cost for 10 years amounts to CHF 1'044'000.

Large samples, e.g., fecal samples, could be delivered and/or stored in aliquoted form.

See appendix 8.2.2 for the cost calculations.

For sample handling and curation of the samples, we estimate that 0.25 - 0.5 FTE (CHF 30'000 - 60'000) have to be allocated.

Regarding the cost for storing lyophilized samples in Svalbard, we estimate lower costs for maintaining the samples, but possibly the setup costs for a facility would be at least as high, if the Global Seed Vault cannot offer storage. This depends on the extent to which facilities accessible from the UNIS University Centre in Svalbard would have to be adapted or newly created. To estimate these costs, further research will be necessary.

Collaboration with local working collections

International shipping costs for samples on dry ice can be in the range of USD 100 or more for a single sample. If samples can be sent in bulk, cost per unit could be reduced. We estimate costs per 1'000 samples in the order of a few 10'000 USD, making this item of expenditure a considerable fraction of the running storage costs.

Costs for characterizing complex samples amount to approximately USD 150 for metagenomic sequencing and USD 300 for metabolomic methods, totaling USD 1'500'000, respectively USD 3'000'000 for a full characterization of 10'000 samples.

Total cost of pilot project

If we consider a pilot cryopreservation facility with a collection of 2'000 samples, metagenomic characterization, and, in parallel, the setting up of a management office for driving the initiative, we estimate that costs in an estimated range of USD 816'000 - 1'210'000 would accrue for two years.

If spare capacity in an existing biobank could be used, a large part of the setup costs of USD 250'000 - 300'000 would not accrue. Likewise, starting with a smaller collection size and not performing metagenomic characterization would reduce costs.

Microbiota Vault Organization	USD 220'000 - 320'000
Setup cryogenic storage facility	USD 250'000 - 300'000
Cryogenic storage	USD 90'000 - 190'000
Sample handling and curation (0.15 - 0.25 FTE)	USD 36'000 - 60'000
Shipping	USD 20'000 - 40'000
Metagenomics	USD 200'000 - 300'000
Total	**USD 816'000 - 1'210'000**
Total (using existing biobank capacity)	**USD 566'000 - 910'000**
Total (using existing biobank capacity, w/o metagenomics)	**USD 366'000 - 610'000**

In a pilot configuration, collections could also be kept frozen at -80 °C, using refrigeration. This would reduce facility setup and storage costs by approximately two thirds.

5.2.7. PEST-Analysis

A PEST-Analysis is a tool used to define and classify external factors that might influence the Microbiota Vault. For this purpose, the analysis divides the factors into four categories: **P**olitical/legal, **E**conomic, **S**ocio-cultural and **T**echnical.

Factor	**Risks**	**Opportunities**
Political/legal		
Legal situation of microbiome	- legal situation unclear: new regulations could promote insecurity - no international legal basis - changing legal situation takes years - microbiome at intersection of different legal systems (human, environment, health)	- legal situation unclear: possibility to intervene and make one's point - new regulations offer new opportunities
International treaty	- drawing-up is time consuming and costly - countries/international organizations not interested in treaty	- include countries that support idea - involve WHO to support idea - be flexible and inclusive in process
Ownership of data	- new regulations could change situation	- open access is on the rise
Biodiversity		- topic is on the rise, could help microbiome
Microbiome research in different countries	- countries not interested	- work with champion countries
Other microbiome biobanks (competitors)	- do not see benefit, work against Microbiota Vault	- support idea, offer network
International reputation of countries	- poor reputation of host country reduces support by other countries	- good reputation of host country promotes support by other countries
Economic		
Funding «climate» (long-term)	- global recession could influence long-term financing of Vault	- hype cycle could boost finances
Finances of microbiome research	- scandal could reduce finances (see gene therapy) - financing biobanks long-term often problematic	- new insights/interesting results could boost finances (clinical trials)
Development of products and therapies in microbiome field (biotech)	- poor products and therapies could influence reputation/finances	- effective products and therapies could add to recognition of the benefits and help finance the project
Industry/big pharma moves into microbiome research (Danone etc)	- poor products and therapies could influence reputation/finances	- effective products and therapies could add to recognition of the benefits and help finance the project

Socio-cultural		
Media	- do not support idea, do not see benefit	- support idea, report on the project
Access and benefit-sharing (NGOs, developing countries)	- NGO criticism damages project - developing countries think they are being exploited	- NGOs can support locally - embracing/stressing access and benefit-sharing as important argument for marketing the initiative in developing countries
General consent/ethical questions	- adds to legal complexity - worldwide general consent difficult	- transparent and clear general consent helps project
Microbiome community	- does not support idea, does not support network	- supports network
Technical		
New technologies (storage, metagenomics, etc.)	- success of long-term storage still unknown	- could facilitate long-term storage, transport, resuscitation - Metagenomics/digital revolution (samples may not be required, only data)
Patents, IP issues	- could slow down microbiome research	- opens ways to new financing sources
Antibiotic Resistances		- urgent and international topic - increase pushes microbiome research

Conclusion PEST-Analysis

This (non-exhaustive) PEST-Analysis shows that many external factors have a major influence on the further development of the project. It is crucial to focus on the factors that can be influenced (at least to a certain extent). For example, the Microbiota Vault project team has little influence on the legal situation of the microbiome worldwide, but could take a more active role in the drafting of an international treaty. Other factors on which the project team can have some influence:

- other microbiome biobanks and the microbiome community (include biobanks and community early on)
- media
- NGOs, developing countries (include NGOs and countries early on)
- awareness building and education of governments that may host Vault sites (seek the policy-level and the international arena early on)

A monitoring of the various factors could help prepare for positive as well as negative developments.

6. Conclusions and proposed model

Based on the analysis and information from the expert interviews, we come to the following conclusions and propose the following model.

6.1. Initially, focus on human samples

In a first phase, the Microbiota Vault should focus on human-body-associated microbiota samples, primarily stool samples. Other source tissues may be considered for the future, but the focus on human samples and human health will facilitate funding the pilot project and not getting lost in complex questions concerning other samples. Environmental samples, e.g., agriculture-associated samples, may be considered. However, it would need to be clearly recognized that the regulatory-political context is fundamentally different.

Additionally, the focus should be on complex rather than axenic samples. A matured Microbiota Vault may take in strains, but complexity and size would be multiplied (durability may be better for cloned strains, but for the moment it remains unclear whether the strains represent the initial diversity).

6.2. Metagenomics are a success factor

Metagenomic characterization will represent an important ingredient for the attractivity of the Vault and will be an important link to the research community. As the Vault intends to preserve complex samples, these will constitute a finite resource whose consumption for research will have to be carefully regulated. Data, however, can be openly available with the consent of the depositor, and hence will be an important success factor. It is conceivable that metagenomic characterization will be repeated on (possibly previously extracted DNA) samples whenever a new analysis technology becomes state-of-the-art.

Metagenomic characterization is likely to be seen as an act of research, tied to legal complexity, which is why we recommend that this procedure be performed by the local working collections. In developing countries, this will be part of a capacity building effort: the Vault Network will either train local labs to conduct the metagenomic characterization using standard protocols and/or offer such characterization through the network or a partner thereof. All samples that go into the Vault should ideally have been characterized (although, if not possible, the priority should be their preservation). All metagenomic data should be made open access, which is an important aspect of benefit-sharing. The Vault must operate under the principles of open access and benefit-sharing (see also the legal considerations in 5.2.3 in the context of the Nagoya Protocol). (Note: from the perspective of developing nations, open access is not sufficient, since it is capacity-reliant).

6.3. Build an international organization and network

To become a global project, the Vault needs to be supported by an international organization on the level of the United Nations (e.g., WHO) or similar. To achieve this, the Vault needs national support to get traction on the international level (similar to CERN with support of Switzerland as a host country). With the "international organization" label, the Vault will achieve credibility above local working collections and beyond national biobanks. This is a prerequisite so that all countries/institutions will send their samples, and will represent an important step in preserving the global microbiota.

To strengthen international collaboration concerning microbiome research, we recommend establishing a network, e.g., "International Microbiota Vault Network" (IMVN). This network will embed the Vault in a research ecosystem and connect the different elements (see Figure 3).

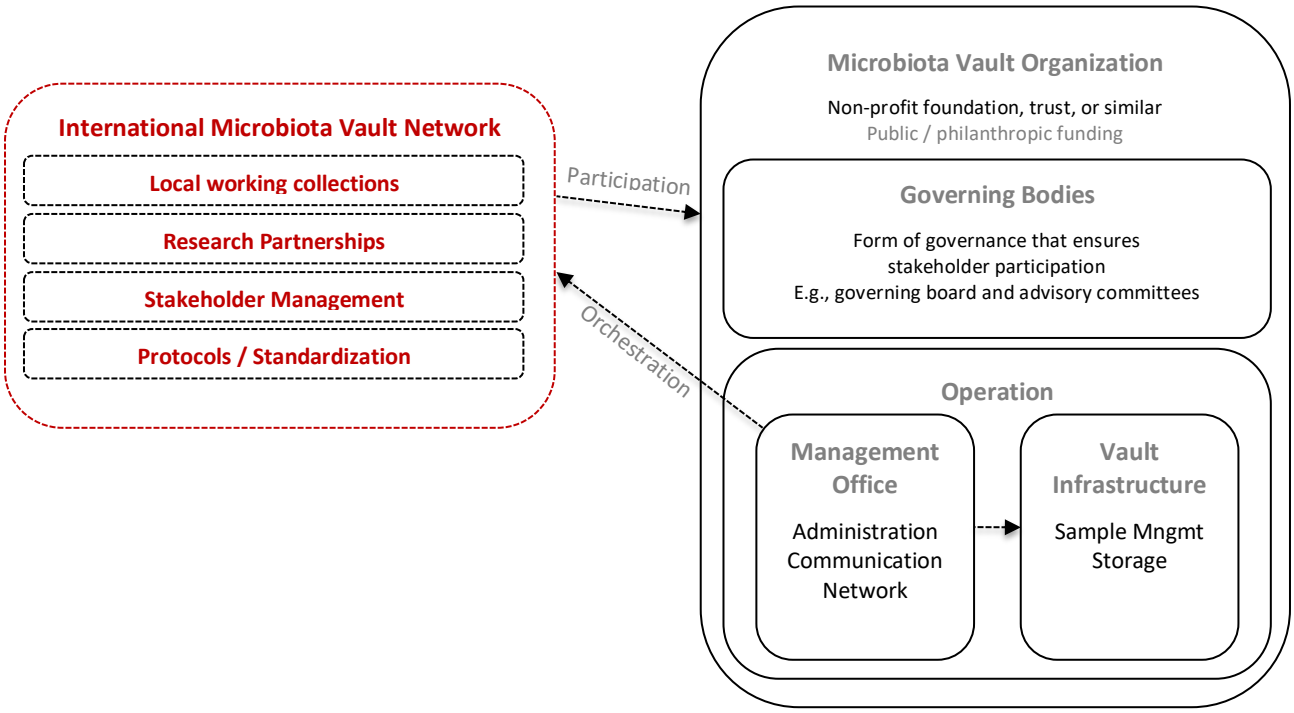

Figure 3: International Microbiota Vault Network

The IMVN will consist of local working collections from all over the globe with a focus on conserving biodiversity. Topics that will be addressed by the network will be partnerships for research, research questions regarding long-term storage and resuscitation, metagenomics and other -omics on Vault samples, and health-directed microbiota research.

The network will be responsible for publishing protocols concerning harvesting, transporting and storing samples. Also, it will have to focus on annotation as well as global standards concerning quality (WHO guidelines for biobanks, etc.). The network will look into legal and ethical frameworks (permits, consent, standards, Helsinki, Nagoya), deal with the fluid legal situation (health/biodiversity) and look into technical standards (data, storability for resuscitation, cultivation and reproducibility).

The network will also be a platform for discussion with stakeholders (politics, WHO, FAO, etc.) and could organize an annual scientific conference to address the above-mentioned issues. The IMVN will be in constant exchange with the Vault Management Office.

Stakeholders for the network:

- Local working collections
- Research community (microbiome, medicine, public health, biodiversity, etc.)
- International organizations (WHO, FAO, etc.)
- Governments (ministries of health, ministries responsible for biodiversity)
- Legal experts
- NGOs
- Media

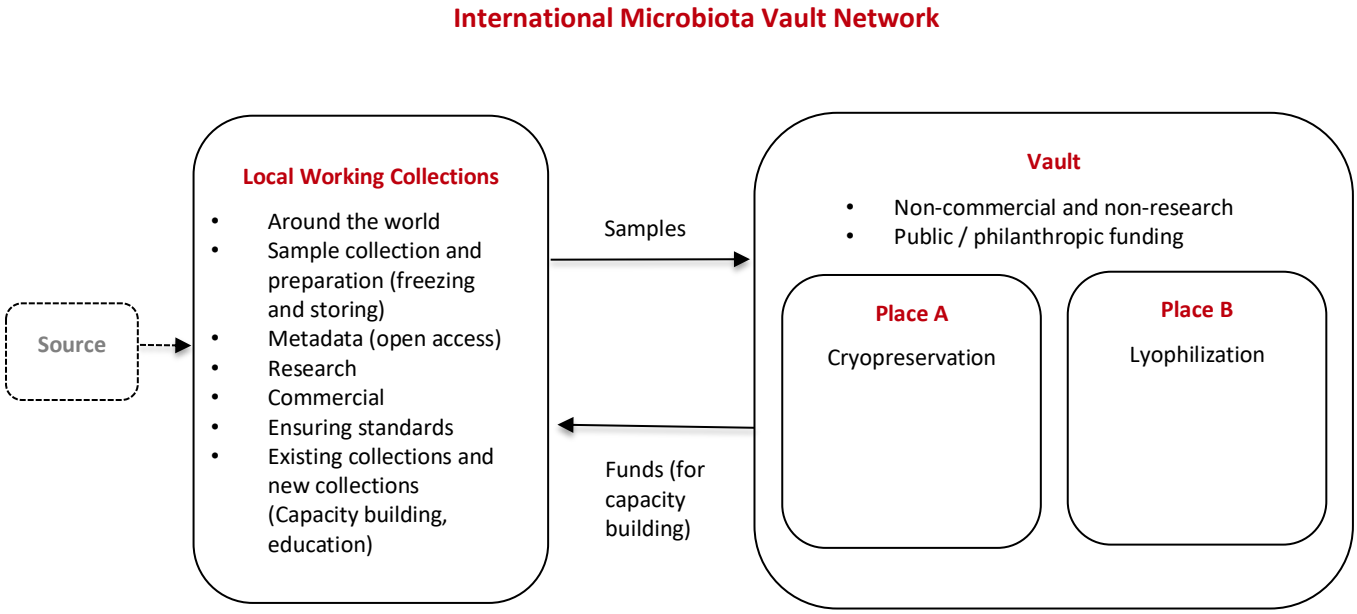

Figure 4: Base Model

Figure 4 illustrates the Base Model of the Microbiota Vault. The idea is to install strategically positioned local working collections around the world that are responsible for collecting and preparing the samples. In developing countries, this would include labs funded by the IMVN in an effort to support capacity building.

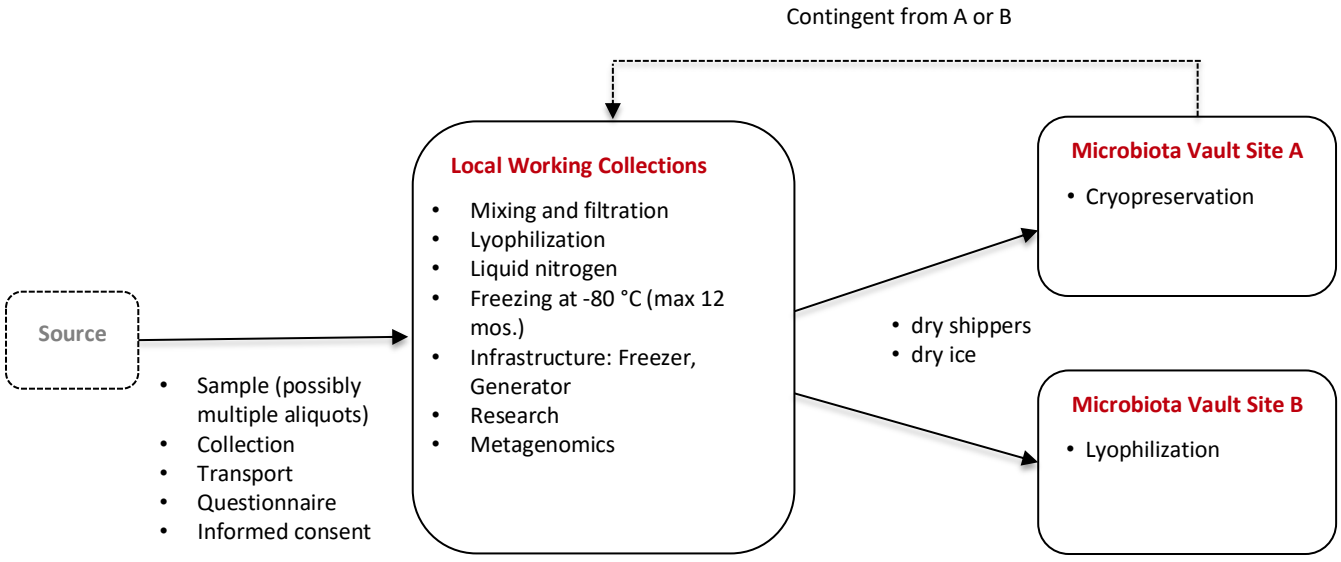

Figure 5: Sample Pathway

Figure 5 shows the sample pathway and goes into some technical details of sample handling. Depending on the emerging policy framework governing the relationship between donor collections and the Vault, there may be a possibility to regain part of a sample from the Vault, but one part will always remain in the Vault (permanent sample).

6.4. Key success factors

- Convince the public - particularly, the non-academic public - of the importance of the microbiome and microbial diversity for human health (establishing the need for a preservation effort)
- Find experts, key stakeholders, organizations, (and finally) countries that support the idea
- Get support from the microbiome research community
- Make the project as simple as possible
- The facility has to be inexpensive to operate (high launching costs and for building the infrastructure, but low annual costs in the long-term)
- Establish a system to conserve genetic diversity (support local and national biobanks)
- Create immediate value for the scientific community by openly sharing digital sample characteristics such as the associated metagenomic sequences (all the while safeguarding privacy by applying appropriate filtering/anonymization techniques)
- Include important stakeholders (politicians, legal experts, NGOs) early on and consult them during the process
- Build a minimal set of ethical standards (e. g., general consent)
- Obtain UN label

7. Recommendations, further actions

Next steps

As a next step, we suggest starting with a pilot project that will include:

- A management office (managing the pilot project, fundraising, communication, development of the International Microbiota Vault Network, further development of the initiative, see also 5.2.2 and 6.3);
- Biobanking infrastructure (in collaboration with local stakeholders at the site);
- Pilot collaboration project with a local working collection in Africa (e.g., Tanzania, Angola) or South America (e.g., Bolivia, Venezuela).

Goals of the pilot project

The pilot project will define transportation, storage and governance details and set up a partnership with a local working collection in a developing country (proof of principle). Ideally, the collection could be set up *de novo*. Preexisting collections could also be used. Optimally, the pilot project could already establish a paradigm for metagenomics, e.g., including a partnership between the Vault, a local working collection, and a research or service partner performing the metagenomics analysis. Such a constellation could form the nucleus for the emerging network. The management office will also discuss sample annotation and establish partnerships with research organizations and foundations.

In parallel, the Vault will need to start a discussion process presenting the overall idea and the details from the feasibility study to stakeholders from academia (including national academies of sciences), governments, authorities, international organizations, etc. This could be done by means of a Chatham House-style discussion. One important result from this process will be the identification of persons, organizations and finally countries that will support the Vault.

Thus, during the pilot phase, both a proof of principle of the Vault model (collecting and safeguarding microbial diversity) can be delivered and the first steps in organizational development taken. Both aspects serve as a basis to position the initiative locally (at the pilot sites) and globally, thereby laying the groundwork to pursue country-level support and to drive the initiative on the international level.

Characteristics of the pilot local collection

- The pilot local collection could entertain existing connections to researchers and/or institutions associated with the Microbiota Vault initiative, making it possible to profit from established forms of collaboration.
- The pilot collection would probably be situated in a developing country, preferably a party to the Nagoya protocol.
- Metagenomic analysis could be included, possibly in partnership with existing research initiatives, thereby demonstrating the network model.

Characteristics of the pilot storage site

- The pilot storage site should be selected according to the criteria described in chapter 5.2.5.
- Pilot storage should be established in collaboration with local stakeholders, including locally established biobanking infrastructures. For Switzerland and Norway, preliminary but strong interest in such collaborations exists.

- Pilot storage should involve cryopreservation as a primary storage mode. Storing of a backup collection of lyophilizates could also occur at this site, since setting up storage of lyophilized samples in a remote arctic location will take longer.

Timeframe and cost

- As estimated in chapter 5.2.6, a two-year duration of the pilot project would consume USD 816'000 – 1'210'000, including infrastructure costs and metagenomic characterization.
- Depending on the setup and choice of storage mode, pilot configurations requiring a smaller budget are conceivable.

8. Appendix

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8.2. Cost calculations

8.2.1. Cryofacility: infrastructure costs

Number	Description	Single Item Price (CHF)	Total (CHF)
10	Tank connections	50	500
200	Piping per meter	950	190'000
10	Data logger	400	4'000
1	Software and servers	25'000	25'000
1	O_2 alarm	1'500	1'500
1	Emergency ventilation	25'000	25'000
1	LN2 management software	5'000	5'000
1	Collection database software	25'000	25'000
Total			**276'000**

Facility capacity: 10'000 samples. Costs without storage tanks. Room rent included in running costs below. The calculation supposes that the facility is built as an extension of an existing biobank.

8.2.2. Cryofacility: sample storage costs

Costs for sample storage, volume 2 ml:

Description	Year 1	Year 2	Year 3	Year 4	Year 5
Number of samples	1’000	2’000	3’000	4’000	5’000
Number of large storage vessels required	1	1	1	1	1
Min. running costs (LN2) per year	11'513.04	11'513.04	11'513.04	11'513.04	11'513.04
General running costs	10'200.00	10'200.00	10'200.00	10'200.00	10'200.00
New equipment (tanks, incl. racks)	49'490.00	-	-	-	-
Total cost estimate (per year)	**71'203.04**	**21'713.04**	**21'713.04**	**21'713.04**	**21'713.04**
Cumulative cost	**71'203.04**	**92'916.08**	**114'629.12**	**136'342.16**	**158'055.20**
Description	Year 6	Year 7	Year 8	Year 9	Year 10
Number of samples	6’000	7’000	8’000	9’000	10’000
Number of large storage vessels required	1	1	1	1	1
Min. running costs (LN2) per year	11'513.04	11'513.04	11'513.04	11'513.04	11'513.04
General running costs	10'200.0	10'200.00	10'200.00	10'200.00	10'200.00
New equipment (tanks, incl. racks)	-	-	-	-	-
Total cost estimate (per year)	**21'713.04**	**21'713.04**	**21'713.04**	**21'713.04**	**21'713.04**
Cumulative cost	**179'768.24**	**201'481.28**	**201'481.28**	**244'907.36**	**266'620.40**

Costs for sample storage, volume 120 ml:

Description	Year 1	Year 2	Year 3	Year 4	Year 5
Number of samples	1’000	2’000	3’000	4’000	5’000
Number of large storage vessels required	1	2	2	3	4
Min. running costs (LN2) per year	11'513.04	23'026.08	23'026.08	34'539.12	46'052.16
General running costs	10'200.00	10'550.00	10'550.00	10'900.00	11'250.00
New equipment (tanks, incl. racks)	67'350.00	67'350.00	-	67'350.00	67'350.00
Total cost estimate	**89'063.04**	**100'926.0**	**33'576.08**	**112'789.12**	**124'652.16**
Cumulative cost (large tank)	**89'063.04**	**189'989.12**	**223'565.20**	**336'354.32**	**461'006.48**
Description	Year 6	Year 7	Year 8	Year 9	Year 10
Number of samples	6’000	7’000	8’000	9’000	10’000
Number of large storage vessels required	4	5	6	6	7
Min. running costs (LN2) per year	46'052.16	57'565.20	69'078.24	69'078.24	80'591.28
General running costs	11'250.00	11'600.0	11'950.00	11'950.00	12'300.00
New equipment (tanks, incl. racks)	-	67'350.00	67'350.00	-	67'350.00
Total cost estimate	**57'302.1**	**136'515.20**	**148'378.2**	**81'028.24**	**160'241.28**
Cumulative cost (large tank)	**518'308.64**	**654'823.84**	**803'202.08**	**884'230.32**	**1'044'471.60**

Storage vessels: Isothermal LN2 freezer CBS V5000-AB.

Costs in CHF, 1 USD = 0.98 CHF as of February 2020

8.3. Landscape of existing projects and initiatives

8.3.1. Microbiota collection projects

Source Google search

Search date August 2019, some updates January 2020

Keywords stool bank; fecal bank; gut microbiome collection; microbiome bank; microbiome collection; microbiome conservancy; microbiome database; microbiota bank; microbiota collection; microbiota conservancy; oral microbiome collection; oral microbiome conservancy; vaginal microbiome conservancy; vaginal microbiome collection

Name	Institution	Country	Type	Goal	Sample type	Size	Meta data	Comments
American Gut	Knight Lab, UC San Diego	USA	microbiome characterization, citizen science initiative	regional project from the Microsetta Initiative, accepts samples from the Asian Gut and Australian gut project	stool, oral, skin	> 21'000 combined from all local initiatives	DNA sequencing	part of the Microsetta Initiative
Armpit Microbes	Dunn Lab, North Carolina State University	USA	research study	investigate what microbes are found in the armpit	armpit	28	16S rRNA	Publication: "Urban J, Fergus DJ, Savage AM, Ehlers M, Menninger HL, Dunn RR, Horvath JE. (2016). The effect of habitual and experimental antiperspirant and deodorant product use on the armpit microbiome. PeerJ 4:e1605 https://doi.org/10.7717/peerj.1605"

Asia Microbiota Bank	Asia Microbiota Bank	Hong Kong	stool bank commercial	fecal microbiota transplant	stool (frozen oral capsule or frozen liquid solution)	information not available	potentially Next Generation Sequencing	
Aussie Gut Project	Griffith University	Australia	microbiome characterization	profile microbiome to provide dietary advice	stool	information not available	information not available	
Belly Button Biodiversity	Dunn Lab, North Carolina State University	USA	research study	investigate what microbes are found in the navel	belly button swab	153 Batch1, 273 Batch 2	16S rRNA	Publication: "Hulcr, J., Latimer, A. M., Henley, J. B., Rountree, N. R.**, Fierer, N., Lucky, A., Lowman, M. D., Dunn RR (2012). A jungle in there: bacteria in belly buttons are highly diverse, but predictable. PLoS ONE 7(11): e47712. doi:10.1371/journal.pone.0047712"
BiomeBank	The Hospital Research Foundation	Australia	stool bank public	fecal microbiota transplant	stool (saline and glycerin, -80 °C)	information not available	information not available	
Brazilian Microbiome Project	Brazilian Metagenomic Consortium (not yet assembled)	Brazil	meta-genomic database (not online yet)	co-ordinate and standardize metagenomic projects in Brazil	environmental, animal, human	0	information not available	Publication: "Pylro, V.S., Roesch, L.F.W., Ortega, J.M. *et al.* Microb Ecol (2014) 67: 237. https://doi.org/10.1007/s00248-013-0302-4". Book Chapter (2017) https://www.springer.com/gp/book/9783319599953#aboutAuthors" most content on website not available yet

British Gut	Knight Lab, UC San Diego	UK	microbiome characterization, citizen science initiative	regional project from the Microsetta Initiative	stool, oral, skin	> 21'000 combined from all local initiatives	DNA sequencing	part of the Microsetta Initiative
Disbiome	Drug Quality and Registration group, University of Ghent	Belgium	meta-genomic database	observe how bacterial composition is altered by disease	feces, saliva, vaginal, urine and others	1’468 organisms	16S rRNA and others	Publication: "Janssens Y, Nielandt J, Bronselaer A, Debunne N, Verbeke F, Wynendaele E, Van Immerseel F, Vandewynckel YP, De Tré G and De Spiegeleer B. Disbiome database: linking the microbiome to disease. BMC Microbiology 2018; 18(1):50."
Earth Microbiome Project	EMP Working Group, EMP Consortium	world-wide	meta-genomic database	collect samples worldwide to construct a global microbial map	environmental, human	27’398 samples including non-human (by 1.11.17)	16S, 18S sequencing	Publication: "Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., …, Jansson, J. K., Gilbert, J. A., Knight, R., & The Earth Microbiome Project Consortium (2017). A communal catalogue reveals Earth’s multiscale microbial diversity. Nature, 551:457-463. doi:10.1038/nature24621."
Flemish Gut Flora Project	VIB	Belgium	research study	example for large-scale fecal sampling, compares different options of user experience and preservation	stool	more than 5’000 individuals	16S rRNA	Publication: "Doris Vandeputte, Raul Y. Tito, Rianne Vanleeuwen, Gwen Falony, Jeroen Raes. Practical considerations for large-scale gut microbiome studies, FEMS Microbiology Reviews, Volume 41, Issue Supp_1, August 2017, Pages S154–S167, https://doi.org/10.1093/femsre/fux027"

Forensics Microbiome Database	J. Craig Venter Institute	USA	meta-genomic database	predict geographical location from human microbiota in forensics	feces, saliva, vaginal, urine and others	20'905 samples	16S rRNA	Publication: "Clarke TH, Gomez A, Singh H, Nelson KE, Brinkac LM. Integrating the microbiome as a resource in the forensics toolkit. Forensic science international. Genetics. 2017-09-01; 30.141-147. PMID: 28728057"
Gentse Stoelgangbank	Ghent University	Belgium	stool bank	fecal microbiota transplant	stool	information not available	information not available	
Global Microbiome Conservatory	GMC Working Group, GMC Consortium	USA	microbiota collection	preserve the human gut microbiome for the benefit of society	bacterial isolates	7'758 bacterial isolates with 3'632 paired genome sequences	genome sequencing	
Gut Microbiota Bank	Gut Microbiota Bank	South Korea	product	commercial online gut microbiota catalogue	gut microbiota	approximately 600 gut microbiota cultures	information not available	species list last updated in 2017
HAMBI	Helsinki Institute of Life Science, University of Helsinki	Finland	microbiota collection	culture collection for teaching and research purposes	mainly environmental, freeze-dried, some active cultures	online catalogue shows 2'941 results	characterization on request	
Human Food Project	Jeff Leach	Africa	research study	find out what a normal microbiome is by examining people with a non-	stool	information not available	16S rRNA	connected with American Gut and metadata is in Earth Microbiome Project

				westernized lifestyle				
Human Gastrointestinal Bacteria Culture Collection (HBC)	Forster, S.C., Kumar, N., Anonye, B.O. *et al.*	UK, USA	microbiota collection	provide cultivated bacteria for studies of the function of the gut biome	bacterial isolates	737 whole-genome-sequenced bacterial isolates	genomic DNA sequencing	Publication: "Forster, S.C., Kumar, N., Anonye, B.O. *et al*. A human gut bacterial genome and culture collection for improved metagenomic analyses. Nat Biotechnol 37, 186–192 (2019) doi:10.1038/s41587-018-0009-7"
Human Microbiome Project	Broad Institute, Baylor College of Medicine, Washington University School of Medicine, J. Craig Venter Institute, DACC	USA	research study	characterize the human microbiome	stool, oral, skin, nose, urogenital	> 11'000 samples	16S rRNA gene analysis via 454 pyrosequencing	project ended in 2013 more information: https://www.nature.com/articles/nature11234#f1
Human Oral Microbiome Database	Chen, T. *et al.*	USA	microbiota collection	find out what bacterial species are present in the oral cavity (HOMD) and the human aerodigestive tract (eHOMD)	oral microbiota	619 taxa in 13 phyla	16S rRNA	Publication: "Dewhirst, F.E., Chen, T., Izard, J, Paster, B.J., Tanner, A.C.R., Yu, W.-H., Lakshmanan, A., Wade, W.G. (2010). The Human Oral Microbiome. J. Bacteriol. 192: 5002-5017." expanded HOMD available (eHOMD)
Human Pan-Microbe Communities Database	Wellcome Sanger Institute, European Bioinformatics Institute	UK	meta-genomic database	support research in basic microbiology, immunology,	gastro-intestinal	5’432 samples	16S rRNA	Publication: "HPMCD: the database of human microbial communities from metagenomic datasets and microbial reference genomes.Forster SC, Browne HP, Kumar N, Hunt M, Denise H, Mitchell A, Finn RD, Lawley

				and therapeutic development				TD.Nucleic Acids Res. 2015 Nov 17. pii: gkv1216"
LifeLines DEEP & DAG3	Lifelines	The Netherlands	research study	study the role of the microbiome in chronic diseases	blood, exhaled air, stool	DEEP: 1'539 participants DAG3: planned 10'000 participants	16S rRNA	Publication: "Tigchelaar EF, Zhernakova A, Dekens JAM, *et al.* Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics BMJ Open 2015;5:e006772. doi: 10.1136/bmjopen-2014-006772"
Microba	Microba	Australia	microbiome characterization	profile microbiome to provide microbiome coaching	stool	information not available	available	
Microbioma	Microbioma	Spain	stool donor bank	platform to connect people for FMTs	stool	>1'000 donors registered	no	only connects donor and recipients, no samples
MICRObiome Among Nurses	Harvard Medical School	USA	research study	how lifestyle and disease influence gut bacteria composition	stool, oral	goal: recruiting 25'000 nurses	16S rRNA	
Microbiome Treatment Centre	University of Birmingham	UK	stool bank	fecal microbiota transplant	stool (fresh, frozen)	>200 completed FMTs	information not available	
Microbiotica	Wellcome Sanger Institute	UK	microbiota collection	characterize and phenotype gut bacteria to link phenotype to function	human microbiota	information not available	available	

Million Microbiome of Humans Project (MMHP)	Karolinska Institutet, Shanghai National Clinical Research Center for Metabolic Diseases, University of Copenhagen, Technical University of Denmark; MetaGenoPolis at the National Institute for Agricultural Research (INRA), Latvian Biomedical Research and Study Centre, Shenzhen BGI Research	China, Den-mark, France, Latvia, Sweden	meta-genomic database	build the world's largest human microbiome database	intestines, mouth, skin, reproductive tract and others	currently 10'000 samples, planned 1M	shotgun metagenomic sequencing	launched recently (26.10.2019) more information: https://news.ki.se/first-project-to-create-atlas-of-human-microbiome
Nederlandse Donor Feces Bank	NDFB Workgroup	The Netherla nds	stool bank	fecal microbiota transplant	stool (fresh, frozen)	information not available	information not available	website only available in Dutch
NHS FMT	Guy's and St Thomas's Hospitals	UK	stool bank	fecal microbiota transplant	stool	information not available	information not available	
OpenBiome	OpenBiome	USA	stool bank nonprofit	fecal microbiota transplant	stool (filtered, sterile dilutant containing glycerol, -80 °C)	35,000 fecal microbiota preparations for FMT	16S rRNA	
Pharmabiome	ETH Zurich SpinOff	Switzer-land	product	microbiota product with	stool	not applicable	no	

				the goal to target inflammatory/ cancerous diseases				
Resistance Surveillance Project	The British Society for Antimicrobial Chemotherapy	UK, Ireland	microbiota collection	antibiotic resistance research program	bacterial isolates from blood and respiratory tract	collection of >50'000 clinical isolates	information not available	
The Biocollective	The Biocollective	USA	stool bank and others	sells collection kits, has a data and sample bank, offers reference strains	stool, bacterial strains	information not available	available	
The Microsetta Initiative	Knight Lab, UC San Diego	USA	microbiome characterization, citizen science initiative	internationally expand the American and British gut project	stool, oral, skin	> 21'000 combined from all local initiatives	DNA sequencing	connected to the Earth Microbiome Project
The Oral Microbiome Bank of China	Xian, P., Xuedong, Z., Xin, X. *et al.*	China	microbiota collection	extend HOMD with information from Chinese study subjects	oral microbiota	289 bacterial strains and 720 clinical samples	16S rRNA	Publication: "Xian, P., Xuedong, Z., Xin, X. *et al.* The Oral Microbiome Bank of China. Int J Oral Sci 10, 16 (2018) doi:10.1038/s41368-018-0018-x"
TwinsUK	King's College London	UK	Research study	UK's largest adult twin registry to investigate the incidence of	blood, urine, stool, saliva	>5'000 stool samples	16S rRNA, for a subset also amplicon sequence	Publication: "Verdi, S., Abbasian, G., Bowyer, R., Lachance, G., Yarand, D., Christofidou, P., . . . Steves, C. (2019). TwinsUK: The UK Adult Twin Registry Update. Twin Research and Human

				multiple diseases				Genetics, 22(6), 523-529. doi:10.1017/thg.2019.65"
uBiome	uBiome	USA	microbiome characterization	track diet and lifestyle by regularly analyzing the microbiome	gut, genitals, mouth, nose, or skin	information not available	16S rRNA	filed for bankruptcy in 2019, FBI investigation over possible insurance fraud Wikipedia article: https://en.wikipedia.org/wiki/UBiome
Vaginal Microbiome Consortium	Virginia Commonwealth University	USA	research study (several)	study the impact of the vaginal microbiome on women's health	mouth, skin, vagina and rectum	> 200'000 samples in MOMS-PI study	16S rRNA, metagenome, metatranscriptome	funded through the Human Microbiome Project
Viome	Viome	USA	microbiome characterization	profile microbiome to provide dietary advice	stool	goal: 2 M	meta-transcriptome sequencing	
Wessex Faecal Microbiota Bank	University of Portsmouth	UK	stool bank	fecal microbiota transplant	stool (fresh, frozen)	information not available	information not available	
World Federation for Culture Collections	World Federation for Culture Collections	world-wide	federation	support and protect culture collections worldwide	mainly environmental	768 culture collections from 76 countries	several databases (CCinfo, WDCM Reference Strain Catalogue, Global Catalogue of Microorganisms)	latest newsletter 2019, conference in 2020 information on other federations: http://www.wfcc.info/collections/networks/

8.3.2. Biodiversity initiatives

Source Google search

Search date August 2019, some updates January 2020

Keywords biodiversity initiatives; biodiversity conservation; WHO biodiversity; UN biodiversity; EU biodiversity

Name	**Goal**
Convention on Biological Diversity	the global legal framework for preserving biodiversity (Cartagena & Nagoya protocol)
Convention on International Trade in Endangered Species of Wild Fauna and Flora	safe international trade of wild animals and plants
EU Biodiversity Strategy	help stop the loss of biodiversity
Food and Agriculture Organization of the United Nations	promote sustainability in social, economic and environmental dimensions
Global Environment Facility	solve the most pressing environmental problems
Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services	strengthen the science-policy interface for biodiversity and ecosystem services
The BioDiversity Conservancy	document what elements of biodiversity are most vulnerable to extinction
The Biodiversity Initiative	conserve biodiversity through ecology, exploration and education

8.3.3. Seed banks, vaults

Source Google search

Search date August 2019, some updates January 2020

Keywords seed vault; seed bank; biodiversity vault; doomsday vault; biodiversity backup; conservation of seeds

Name	Location	Size
AVRDC - The World Vegetable Center	Shanhua, Taiwan	more than 61'235 accessions of 440 species from 151 countries
Bioneers Seed Saving Initiative	Yearly conferences in the USA	information not available
Camino Verde	Concord, Massachusetts and Puerto Maldonado, Peru	around 400 species of trees
Chang La Vault	Chang La, India	10'000 seed samples and 200 plant species
Hawai'i Public Seed Initiative	Kamuela, Hawaii	information not available
International Center for Tropical Agriculture	Palmira, Colombia	67'700 crop samples
International Potato Center	Lima, Peru	over 11'000 accessions
Louisiana Native Plant Initiative	Louisiana, USA	information not available
Millennium Seed Bank Partnership	Wakehurst, England	over 10 percent of all plant species
National Gene Bank by Agroscope	Vaud, Switzerland	13'000 accessions
Native Seed / SEARCH	Arizona, USA	approximately 1'900 different accessions of traditional crops
Navdanya	Uttrakhand, India	120 community seed banks in 17 states of India
New York City Native Plant Conservation Initiative	New York City, USA	information not available
Planned: Productive Landscapes	Tanzania	information not available
Seed Savers Exchange	Iowa, USA	20'000 different varieties of heirloom and open-pollinated plants
Svalbard Global Seed Vault	Svalbard, Norway	5'997 species
The NSW Seedbank	Sydney, Australia	many of the 25'000 plant species that occur in Australia
U.S. Government Seed Banks	20 locations in the USA	close to 600'000 different varieties