

Preliminary Characterization of the American Gut Population

As of July 04, 2014, we have completed sequencing and analysis of gut, skin, and oral bacteria from the first 3,812 samples of 3,232 participants in the American Gut study. This document gives us a summary of the most up-to-date results for the whole population.

Here we compare the American Gut population to other populations who have had their gut, oral, and skin bacteria characterized. We then describe the participants in the study, show the major kinds of bacteria in the gut microbiota of the American Gut population, and provide some information about what affects gut bacteria (as well as show that some variables such as gender have surprisingly little effect).

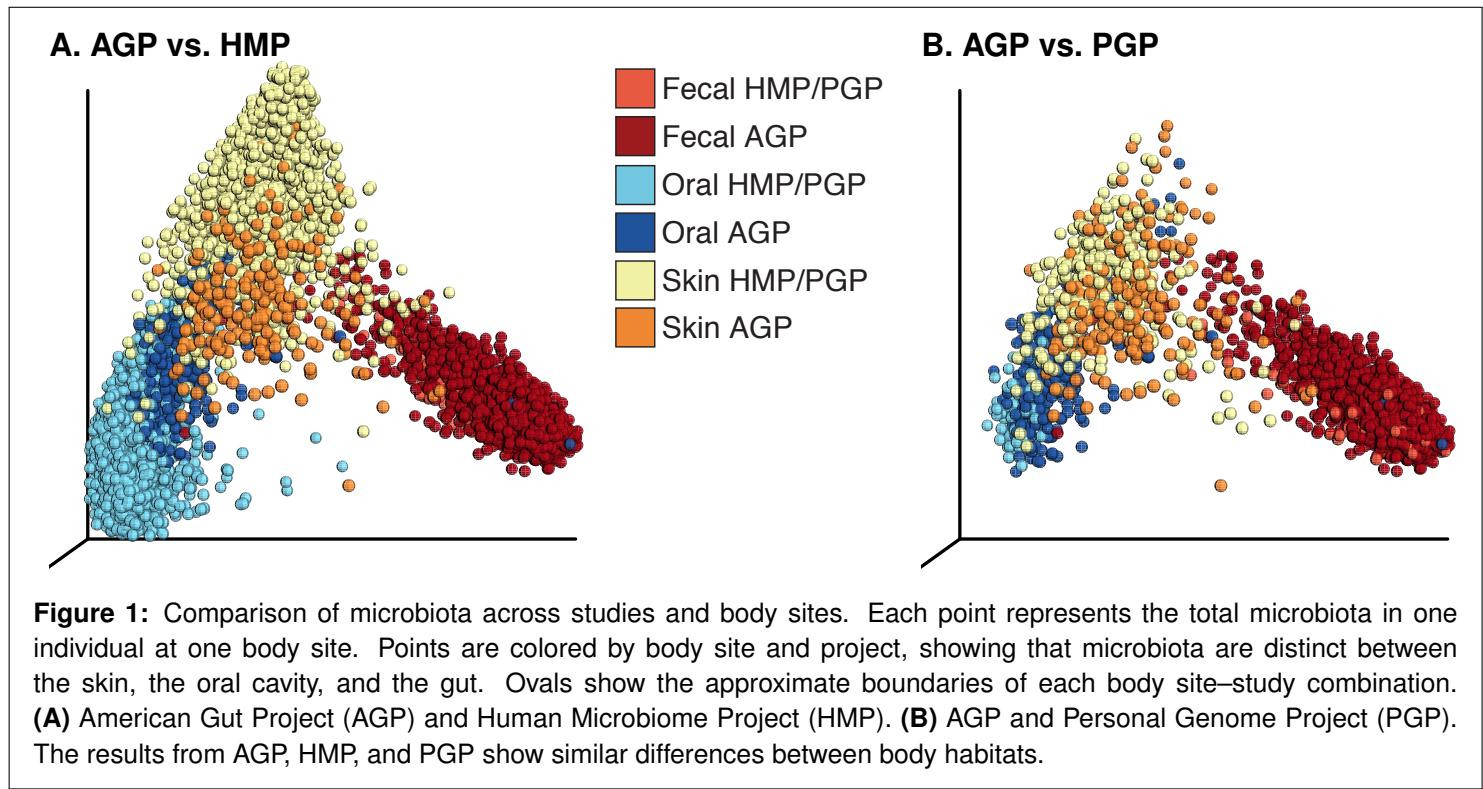
Technical note: “microbiota” refers to a particular community of microbes, including bacteria (e.g., the human gut microbiota); “microbiome” refers to the genes those microbes contain (e.g., the human gut microbiome). Most participants in American Gut have signed up for characterization of the microbiota.

A map of your microbes

A useful way to compare thousands of microbiota samples at the same time is by putting them on a map where more similar samples are closer together, and more dissimilar ones are further apart.

Figure 1A shows how the microbes from different parts of the body relate to one another: as you can see, the fecal (gut), oral and skin microbiota form three points of a triangle, reflecting the major differences between these body locations. (Amazingly, even the same person has essentially none of the same bacteria in their skin, their mouth, and their gut.) In these figures, we compare the data from American Gut to the data from the Human Microbiome Project, a \$173 million initiative funded by the National Institute of Health (NIH) to characterize both the microbiota and the microbiome of 300 healthy subjects. Broadly, the American Gut data are very comparable to the Human Microbiome Project data, and to several other projects.

Figure 1B highlights the data from participants in the Personal Genome Project, who have agreed to make their genome data publicly available and who have also had their microbes characterized in the mouth, feces and skin through American Gut.



Who is participating in the AGP?

Our participants

We have sequenced 3,812 microbiota samples from 3,232 people, including 231 participants in the Personal Genome Project. We are just getting started, but already this number substantially exceeds the number of participants in other recent high-profile studies, including the Human Microbiome Project and the Global Gut study, which were published in the same issue of the scientific journal *Nature* last year.

Table 1: Samples and participants in the American Gut project (AGP) compared to the Human Microbiome Project (HMP), Global Gut project (GG), and the Personal Genome Project microbiota component (PGP).

| | HMP | GG | PGP | AGP |
|--------------------|------------|------------------------|------------|------------------|
| Subject Age | Adults | Adults, Children | Adults | Adults, Children |
| Subject Location | USA | Venezuela, Malawi, USA | USA | Global |
| Total Samples | 4,788 | 531 | 1,007 | 3,812 |
| Total Participants | 242 | 531 | 231 | 3,232 |
| Sequences | 36,797,226 | 1,093,740,274 | 23,763,070 | 101,435,579 |

HMP – The Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome. 2012. *Nature* 486: 202-214.

GG – Global Gut, Yatsunenko et al. 2012. Human gut microbiome viewed across age and geography. *Nature* 486: 222-228.

PGP – Personal Genome Project, participants and samples being continually added into the American Gut Project (unpublished data).

AGP – American Gut Project, participants and samples being continually added.

Figure 2: Comparison of gender, age and BMI across projects that characterize the human microbiota. Relative to the HMP, AGP contains more females, far more older and younger people, and a greater number of obese people. The concentration of older people is important because many health issues, including those that affect the gut, only appear later in life.

| | | | |
|-----|-------------------------------|-----|-------------------------------------|
| VSO | Very severely obese (BMI >40) | N | Normal (BMI 18.5–25) |
| SO | Severely obese (BMI 35–40) | U | Underweight (BMI 16–18.5) |
| MO | Moderately obese (BMI 30–35) | SU | Severely underweight (BMI 15–16) |
| O | Overweight (BMI 25–30) | VSU | Very severely underweight (BMI <15) |

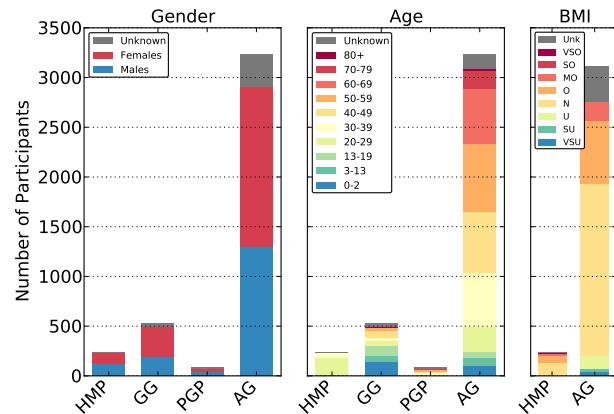
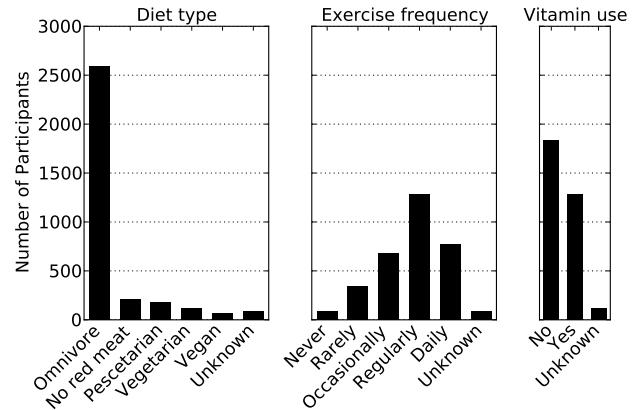


Figure 3: Diet and exercise regimes of the AGP population. According to self-reported data, the majority of our participants are omnivores who exercise frequently. Roughly half use vitamins regularly. As additional participants with more varied diet and exercise characteristics join the study (e.g., more vegans or more people who never exercise), we will be able to say more about how different lifestyles affect gut microbes and perhaps shed light on which lifestyle factors are associated with healthier gut bacteria.



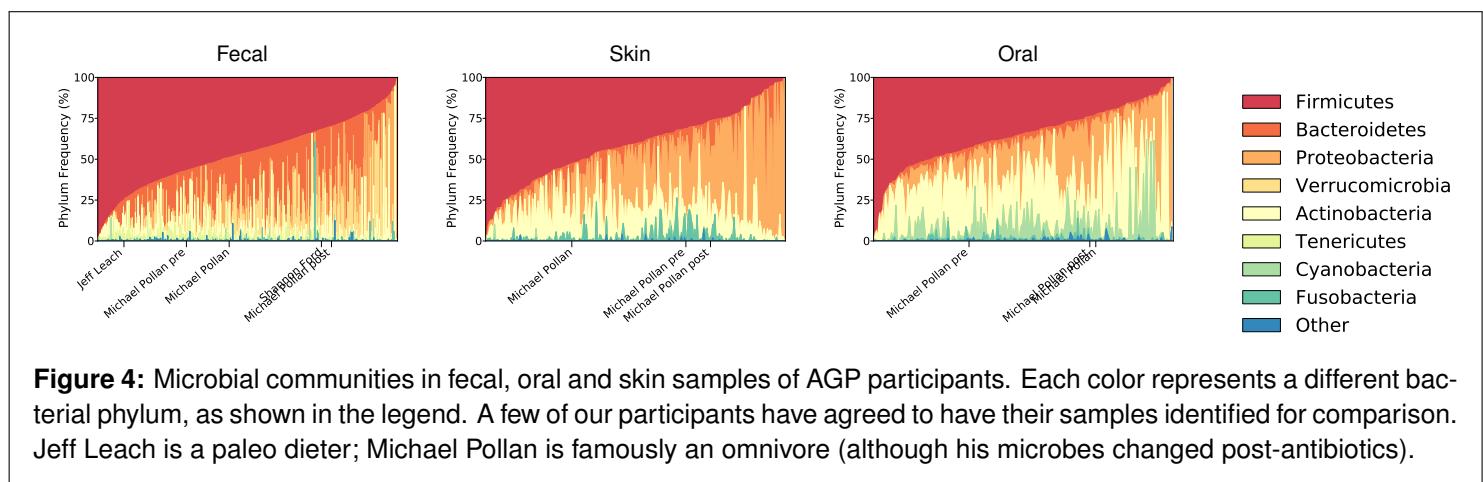


Who is living in your gut?

What kinds of microbes are in different sample types?

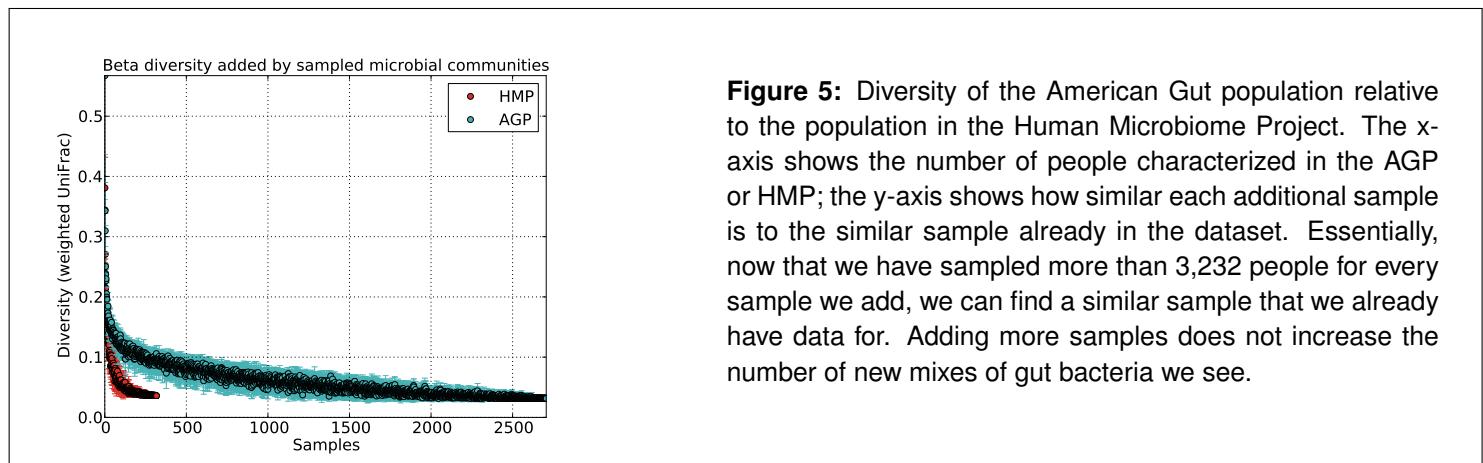
Just as seeing the major types of plants and animals (say, oaks versus pine trees) can give you a broad idea of what a traditional ecosystem looks like, knowing who is in your gut or mouth or skin provides a useful guide to what is going on in there. Different parts of the body have very different microbes, as shown above in **Figure 1**, but also at the same body site, different people have very different microbes. For example, some people have over 90% Firmicutes in the gut, whereas other people have less than 1%. Understanding the reasons for these differences is a major goal of the American Gut project.

The figures below show results at the level of bacterial phyla: a phylum is a big branch on the evolutionary tree of life. (For context, all arthropods, from shrimp to spiders, belong to a single phylum.) Many bacteria can be present in the gut, but not all are present in great numbers. For example, *E. coli* is thought of as a classic gut bacterium because it is good at growing on a petri dish, not because it is a main component of the gut. At the phylum taxonomic level, the major players are Firmicutes and Bacteroidetes. Firmicutes include *Clostridium* and *Lactobacillus* species and many producers of the short-chain fatty acid butyrate. Bacteroidetes includes *Bacteroides* and *Prevotella*, both of which break down polysaccharides (big sugars).



How much have we seen of the American Gut?

The gut is a complex ecosystem, far more like a rainforest than a desert. Additionally, every person has a different mix of microbes in their gut. Therefore, to truly understand how many kinds of microbes there are, we have to sample many people. The AGP has sampled many more people than any other project, including the HMP. Therefore, for the types of people that participate in the AGP, we understand the range of kinds of microbes that exist. Now we just need to understand why and what factors affect them.



What affects your gut microbes?

Factors that affect your gut habitat

As we saw in the last section, different people differ dramatically in what lives in their gut. Many factors, including antibiotic use, are known to change the composition of gut microbiota. But which of the many possible factors has the largest effect? Here we examine the effects of several factors that might affect your gut bacteria.

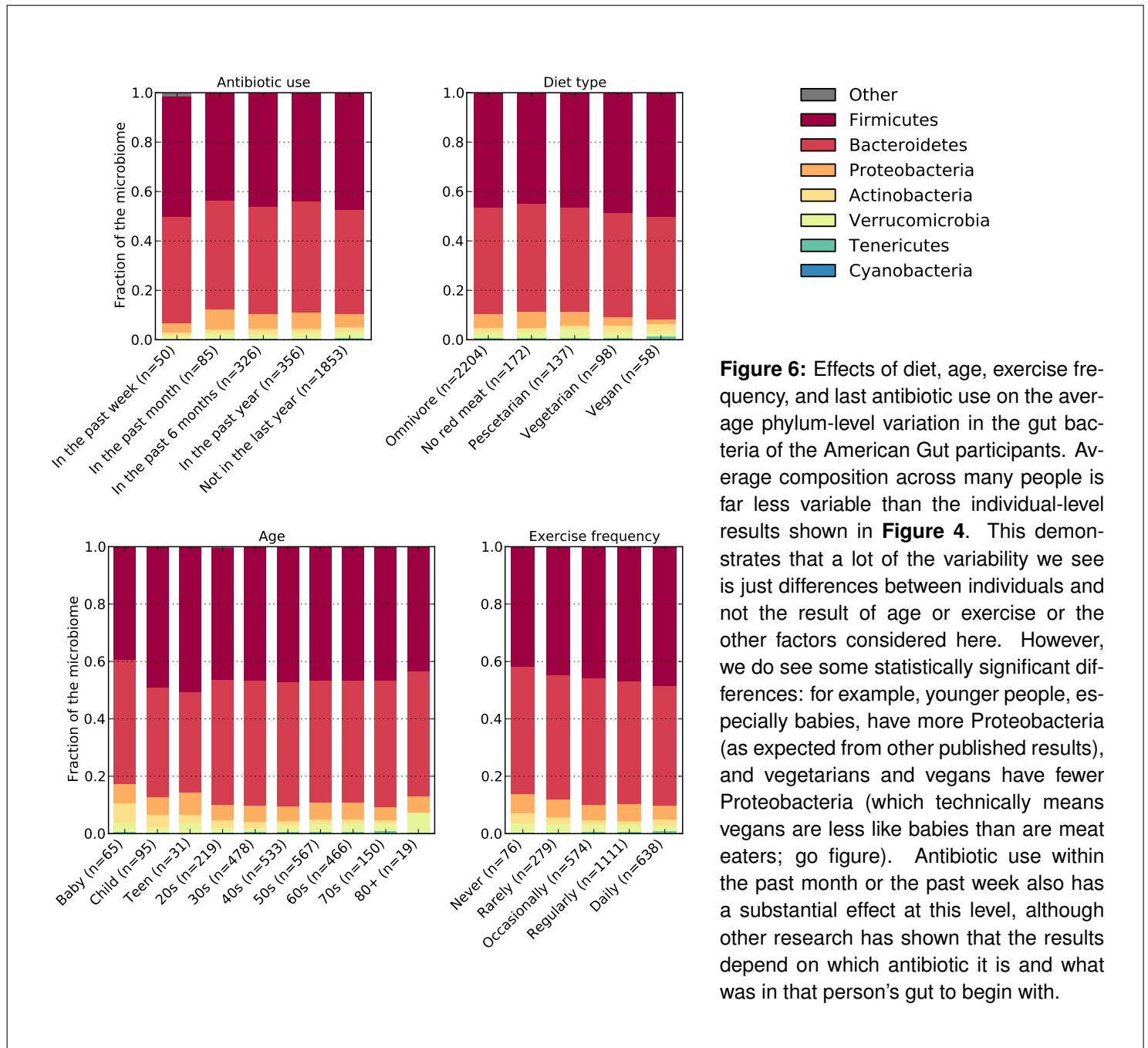


Figure 6: Effects of diet, age, exercise frequency, and last antibiotic use on the average phylum-level variation in the gut bacteria of the American Gut participants. Average composition across many people is far less variable than the individual-level results shown in **Figure 4**. This demonstrates that a lot of the variability we see is just differences between individuals and not the result of age or exercise or the other factors considered here. However, we do see some statistically significant differences: for example, younger people, especially babies, have more Proteobacteria (as expected from other published results), and vegetarians and vegans have fewer Proteobacteria (which technically means vegans are less like babies than are meat eaters; go figure). Antibiotic use within the past month or the past week also has a substantial effect at this level, although other research has shown that the results depend on which antibiotic it is and what was in that person's gut to begin with.

What affects gut bacteria in different studies?

Where do you fit in?

By comparing our results to studies that include participants from other parts of the world, we can see where our American Gut fits into the global picture. People from different countries tend to have different gut microbes, probably due to distinct diets. However, there are also factors, like age, that impact gut bacteria similarly, regardless of what country a person lives in. Comparing our results to Yatsunenko et al. (2012), it is clear we have greatly expanded our knowledge of the diversity within the US population, though there remains much novel diversity to capture in other populations. As such, it is critical to proceed forward in ways that can better foster international participation. To that end, the American Gut Project is forging international partnerships that will bring the project closer to more people, fostering a better understanding of what it means to be human.

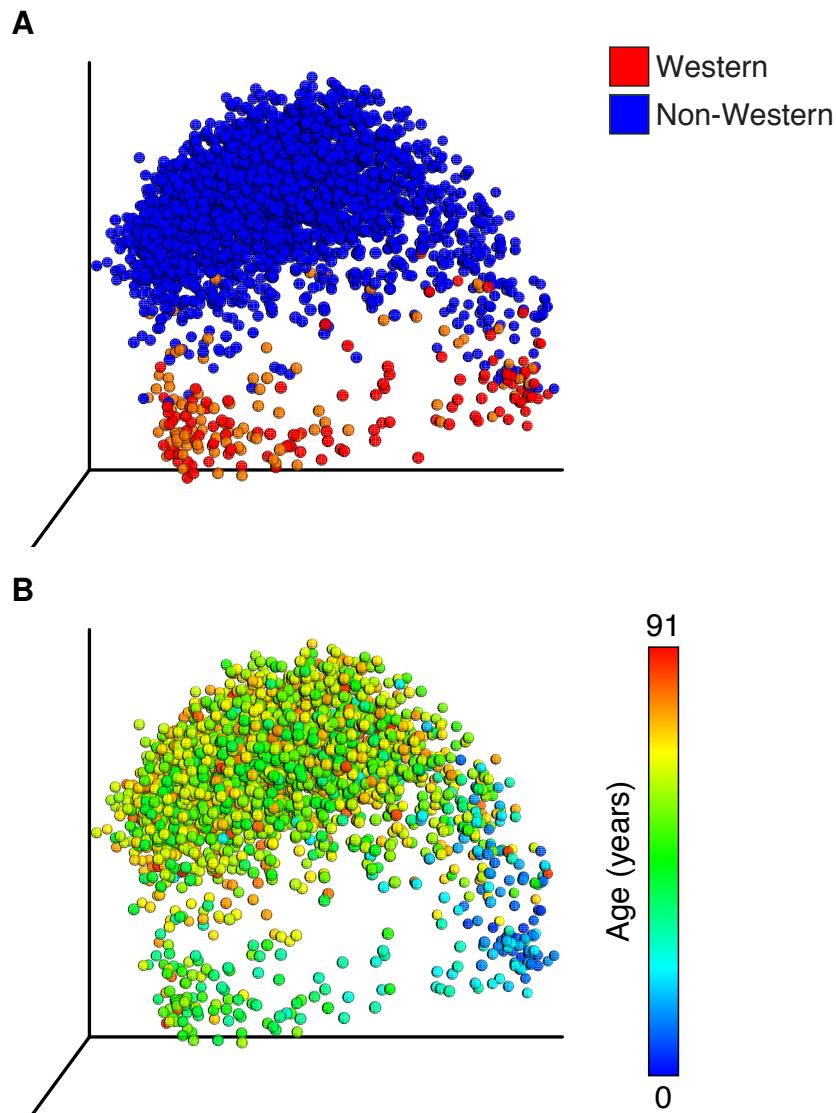


Figure 7: Gut sample diversity by country and age of subjects. **(A)** Western and non-Western guts are largely distinct, possibly owing to the higher consumption of processed sugars and carbohydrates in the Western diet. Here we included Global Gut samples (US, Malawi, Venezuela) and just the US-derived American Gut samples. When we previously examined the Global Gut samples, the US samples were very different from the Venezuela and Malawi samples. Now adding thousands more US samples from the AGP, we observe the same distinction. **(B)** Age has a large effect, with infants (in blue on the righthand side of the diagram) having very different gut microbiota from adults.

The American Gut Project would like to thank . . .

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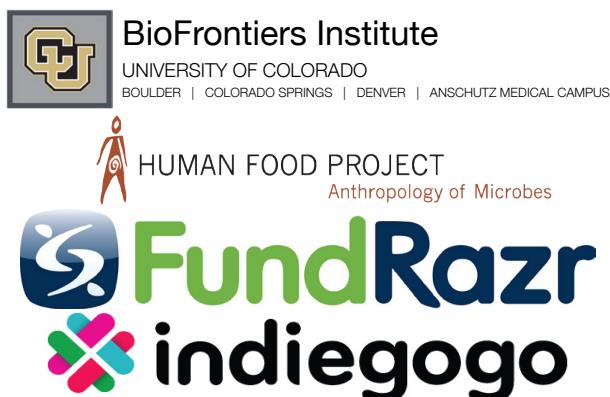
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If you haven't joined American Gut please consider doing so – we need your poo! Join the 6,000+ other participants from around the world that have already donated to this unprecedented study to map the diversity of the American (Global) Gut. Go to www.humanfoodproject.com/american-gut/ and follow the links. For more info email us at info@american-gut.org.

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