

CASE STUDY · MICROBIOME · GUT-BRAIN AXIS

Following the gut–brain chain: what a probiotic changes, and how the host responds

How Microbiome Xplorer combines standardised sampling, strain-resolved metagenomics, metatranscriptomics, metabolomics and host markers to show which communities change, which functions they carry and how the host responds, an illustrative study.

Illustrative case study. This scenario shows how Microbiome Xplorer is applied to a gut–brain question. It does not describe a specific client project or product: study size, results and timelines are indicative and depend on the intervention, the matrices and the study design. Microbiome Xplorer is a research service; it does not diagnose or treat any condition.

1 Sample

Gut, oral, skin, vaginal or any matrix, collected and stabilised to standard

2 Communities

Which taxa and strains change, in absolute terms

3 Functions

Which functions they carry, and which are switched on

4 Host

Which signals reach the host, and what it means

At a glance

QUESTION

Does a probiotic act on the gut–brain axis: and through which microbial functions and host signals?

CLIENT PROFILE

Nutrition company running a randomised, placebo-controlled trial of a three-strain probiotic

PARTICIPANTS

120 healthy adults with moderate perceived stress; 8 weeks of intake + 4-week washout; 112 completers

MATRICES

Stool (4 timepoints), saliva (oral microbiome, cortisol), blood

LAYERS

Strain-resolved shotgun metagenomics, metatranscriptomics, 16S (saliva), stool and plasma metabolomics, host markers; WGS of the product strains

VOLUME

≈1,200 molecular profiles

OUTCOME

Transient engraftment; butyrate, GABA and indole functions ↑; tryptophan shifted towards indoles; no change in inflammation or cortisol; modest stress effect, mediation exploratory

DURATION

≈ 14 weeks from last sample to final report

The challenge

A nutrition company had developed a three-strain probiotic for stress and well-being and was running a randomised, double-blind, placebo-controlled trial in 120 healthy adults with moderate perceived stress. The

clinical endpoints were in place. What was missing was the mechanism: does the product actually act through the microbiota–gut–brain axis¹, and how?

- **Is the product there?** Detecting more Lactobacillus or Bifidobacterium does not prove that the product strains engrafted.
- **Does anything change beyond the strains?** In community composition, in microbial functions, in what reaches the host?
- **Is the story defensible?** Mechanistic data support scientific communication and future dossiers, in the EU, health claims on foods require EFSA assessment², but only if each link is shown, not assumed.

The brief: show which communities change, which functions they carry and how the host responds, and where the chain breaks.

01 **Sample: the right matrices, collected the right way**

The trial was the client’s; the sampling design was built with them before the first participant was enrolled. Each matrix was chosen for the question it answers: stool for the gut community and its products, saliva for the oral microbiome and the cortisol awakening response, and blood for circulating metabolites and inflammatory markers.

TABLE 1. ANY MATRIX YOUR STUDY CALLS FOR: AND WHAT IT CAN TELL YOU			
Matrix	Samples and methods	Key considerations	Links to health under study
Gut	Stool, rectal swabs, mucosal biopsies; shotgun metagenomics, metatranscriptomics, metabolomics	Stabilise at collection; record stool consistency and diet; quantify absolute abundance	Obesity ³ , cardiovascular risk via TMAO ⁴ , mood and stress ¹ , neurodegeneration ⁵
Oral	Saliva, dental plaque, tongue swabs; 16S or shotgun	Standardise timing relative to meals and tooth-brushing	Periodontal health; links with cardiovascular and neurodegenerative disease under investigation ⁶
Skin	Swabs, tape strips; 16S/ITS or shotgun	Low biomass: blanks and contamination controls are essential ⁷	Inflammatory skin conditions, ageing, cosmetic claims ⁸
Vaginal	Self- or clinician-collected swabs; 16S or shotgun	Classify community state types (Lactobacillus dominance) ⁹ ; record cycle phase	Women’s health: bacterial vaginosis, pregnancy, menopause
Other	Nasal, breast milk, environmental samples, animal and in vitro models	Matrix-specific extraction and controls	Respiratory health, infant development, One Health

Pre-analytics decide what the data can show. Participants collected stool at home into a DNA/RNA-stabilising buffer, with the Bristol stool scale and a 3-day food record at each visit; antibiotic use in the previous 3 months was an exclusion criterion. Every extraction batch carried blanks and a mock community, each participant’s samples were processed together, and arms were balanced across

batches¹⁰. Total bacterial load was measured by qPCR so that changes could be expressed in absolute, not only relative, terms¹¹.

02 Which communities change

Microbiome Xplorer combines layers according to the question. Table 2 shows the options and how each was used here.

TABLE 2. FROM “WHO IS THERE” TO “HOW THE HOST RESPONDS”

Layer	Question it answers	In this study
16S rRNA / ITS amplicon sequencing	Who is there? Bacterial and fungal composition at scale; suited to large or low-biomass sample sets	Saliva, weeks 0 and 8
Shotgun metagenomics	Which species and strains, and which functions could they perform? Strain tracking, functional potential, resistome ¹²	All stool samples (4 timepoints)
Metatranscriptomics	Which functions are actually expressed?	Stool subset, weeks 0 and 8 (n = 40)
Metabolomics	Which microbial products reach the host? Short-chain fatty acids ¹³ , tryptophan and indole metabolites ¹⁴ , bile acids, TMAO	Stool and plasma, weeks 0 and 8
Host response	How does the host respond? Inflammatory and barrier markers, hormones; host transcriptomics or proteomics when needed	CRP, IL-6, LBP; salivary cortisol awakening response
Strain genomics (WGS)	Is the product what it should be? Strain identity and absence of acquired antimicrobial-resistance genes ¹⁵	The 3 product strains

In total, ≈1,200 molecular profiles: 448 stool metagenomes, 80 metatranscriptomes, 224 saliva microbiomes, and 448 stool and plasma metabolomes.

- **Longitudinal, compositional statistics.** Mixed-effects models (arm × time, participant as random effect) and bias-corrected compositional differential abundance (ANCOM-BC¹⁶).
- **Confounders in the model.** Stool consistency, diet, BMI and medication, among the largest drivers of gut microbiome variation¹⁷, entered as covariates.
- **Strain-level tracking.** Product strains were distinguished from resident relatives by strain-specific markers, so engraftment means the product itself, not more of the same species.

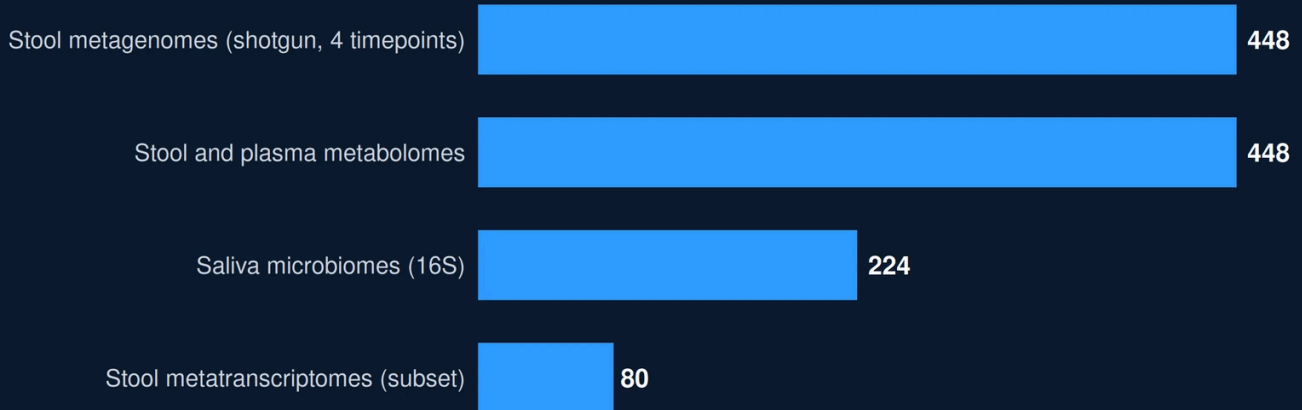
Results (probiotic vs placebo; indicative):

- **Overall diversity stable.** Alpha diversity did not change (Shannon, $p = 0.62$); community composition shifted slightly in the probiotic arm (PERMANOVA $R^2 = 0.8\%$, $p = 0.03$), a small effect, as expected in a healthy adult gut.
- **Butyrate producers up.** Faecalibacterium and Coprococcus, genera previously associated with quality of life¹⁸, rose in absolute abundance (+21% and +17%, $q < 0.05$).
- **Engraftment is real, and transient.** Product strains were detected in 82% of probiotic-arm participants at week 8 and in 9% after the 4-week washout; never in the placebo arm.

- **Engraftment varies between people.** It was lower when closely related resident strains were already present at baseline, consistent with person-specific colonisation resistance¹⁹.
- **Oral microbiome unchanged.** Saliva communities did not shift (PERMANOVA $p = 0.71$): the effect was gut-specific.

What was measured

≈1,200 molecular profiles from 112 completers

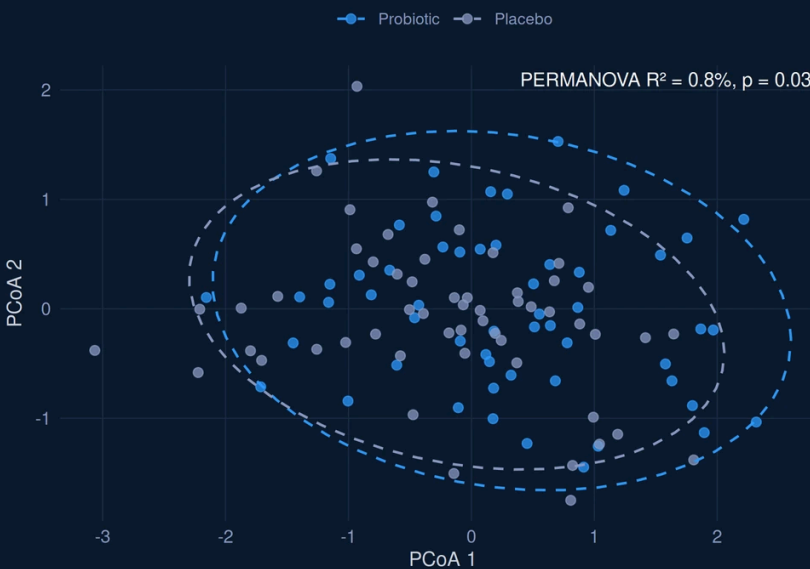


Indicative volumes.

Figure 1. What was measured. Number of molecular profiles generated per layer, about 1,200 in total for 112 participants who completed the trial: stool metagenomes at four timepoints, stool and plasma metabolomes, saliva microbiomes, and stool metatranscriptomes for a subset of 40 participants at weeks 0 and 8. Indicative volumes.

Community composition, week 8

PCoA, Bray-Curtis



Simulated data matching the values reported in the case study.

Alpha diversity

Shannon index, week 8

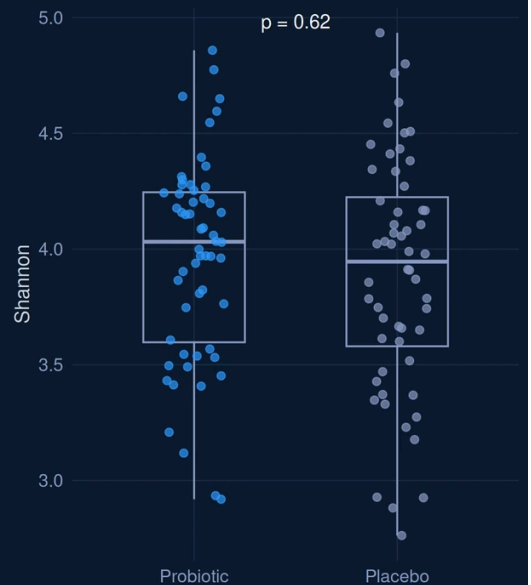


Figure 2. Community composition and diversity at week 8. Left: each point is one participant's gut community, placed so that similar communities sit close together (PCoA on Bray-Curtis distances). The two arms largely overlap: the probiotic shifts overall composition only slightly (PERMANOVA $R^2 = 0.8\%$, $p = 0.03$), as expected in a healthy adult gut. Right: Shannon diversity does not change ($p = 0.62$). Simulated data matching the case study.

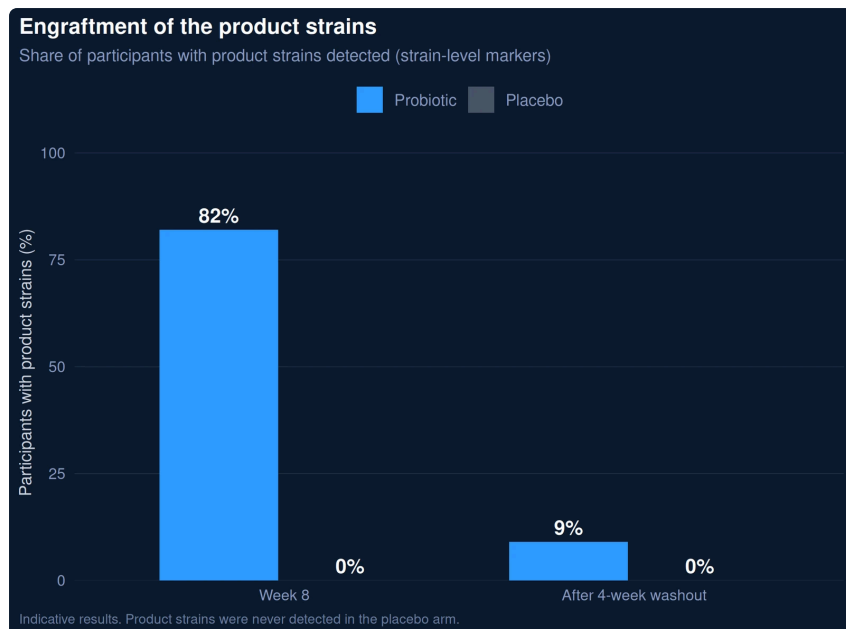


Figure 3. Engraftment of the product strains. Share of participants in whom the product strains were detected, using strain-specific markers that tell them apart from related resident strains. 82% of the probiotic arm carried them at week 8, only 9% four weeks after stopping, and no one in the placebo arm: engraftment is real but transient. Indicative results.

03 Which functions they carry

Species names say who is there; functions say what they can do. Metagenomes and metatranscriptomes were mapped to curated gut–brain modules, microbial pathways that produce or degrade neuroactive compounds¹⁸.

- **Butyrate synthesis: present and switched on.** The module rose in metagenomes (+18%) and, more strongly, in metatranscriptomes ($\times 1.5$).
- **GABA and indoles.** Expression of glutamate decarboxylase (GABA synthesis) and of tryptophanase/indole pathways increased ($\times 1.4$ and $\times 1.3$); serotonin- and dopamine-related modules did not change.
- **Safety checked.** The resistome did not expand, and whole-genome sequencing found no acquired resistance genes in the product strains.

04 How the host responds

A microbiome change matters only if the host responds. Each link of the chain, from product to perceived stress, was tested rather than assumed.

IN THE GUT

1 Product strains engraft

Detected in 82% at week 8

9% after washout

Supported · transient



2 Community shifts

Small overall shift (R^2 0.8%)

Butyrate producers ↑

Modest



3 Functions switch on

Butyrate, GABA, indole pathways ↑ (DNA and RNA)

Supported

IN THE HOST

4 Metabolites reach the host

Faecal butyrate ↑ 15%

Plasma IPA ↑

Kynurenine/tryptophan ↓

Supported



5 Host signalling

CRP, IL-6, LBP unchanged

Cortisol response n.s.

Not shown



6 Perceived stress

PSS −1.6 vs placebo

~24% mediated by butyrate

Exploratory

Figure 4. The gut-brain chain, link by link. Each box is one link between the product and perceived stress, with its evidence and a verdict. The first four links are supported, from engraftment to metabolites reaching the circulation. Host signalling (inflammation, cortisol) did not change, so the final link to perceived stress remains exploratory. Dashed arrows mark links not demonstrated at this dose and duration. Indicative results.

- **Microbial products reach the host.** Faecal butyrate rose by 15% (p = 0.02) and plasma indole-3-propionic acid by 12%; the plasma kynurenine/tryptophan ratio fell by 9%.
- **Inflammation and stress hormones did not move.** CRP, IL-6 and LPS-binding protein were unchanged; the cortisol awakening response fell by 6% (95% CI –14% to +2%).
- **A modest clinical signal.** The client’s primary endpoint, perceived stress (PSS-10), improved by 1.6 points more than placebo (95% CI 0.1–3.1); sleep quality did not differ.
- **Exploratory mediation.** The change in faecal butyrate statistically mediated an estimated 24% of the PSS effect (95% CI 2–51%), a hypothesis to test, not proof.

Interpretation. The data support a coherent but partial mechanism. The strains engraft transiently and raise the community’s capacity, and actual activity, for butyrate, GABA and indole production; these products reach the circulation and shift tryptophan metabolism away from the kynurenine branch. The host signals expected downstream, systemic inflammation and the cortisol response, did not change at this dose and duration. The gut-to-host link is established; the host-to-brain link is not yet.

TABLE 3. WHAT THE DATA SUPPORT		
Statement	Evidence	Verdict
“Product strains colonise the gut during intake”	Strain-level detection in 82% at week 8; 9% after washout	Supported: transient
“Increases the microbiota’s butyrate production”	Metagenome ↑, metatranscriptome ↑, faecal butyrate ↑	Supported: 3 layers
“Shifts tryptophan metabolism towards indoles”	Plasma IPA ↑; kynurenine/tryptophan ↓; tryptophanase expression ↑	Supported: 2 layers
“Gut-specific, without disturbing the resident microbiome”	Oral microbiome and resistome unchanged; no acquired resistance genes in the strains	Supported
“Reduces systemic inflammation”	CRP, IL-6, LBP unchanged	Not supported
“Dampens the cortisol stress response”	–6%; 95% CI –14% to +2%	Not supported
“Reduces perceived stress via the microbiome”	PSS –1.6 vs placebo; ~24% mediated by butyrate (exploratory)	Hypothesis: to confirm in a dedicated trial

In the EU, health claims on foods and food supplements must be authorised under Regulation (EC) No 1924/2006 after EFSA assessment of human efficacy data. Mechanistic microbiome data strengthen the scientific rationale, trial design and scientific communication; they do not by themselves establish a claimed health effect.

Faecal butyrate over the trial

Mean and 95% CI, baseline = 100

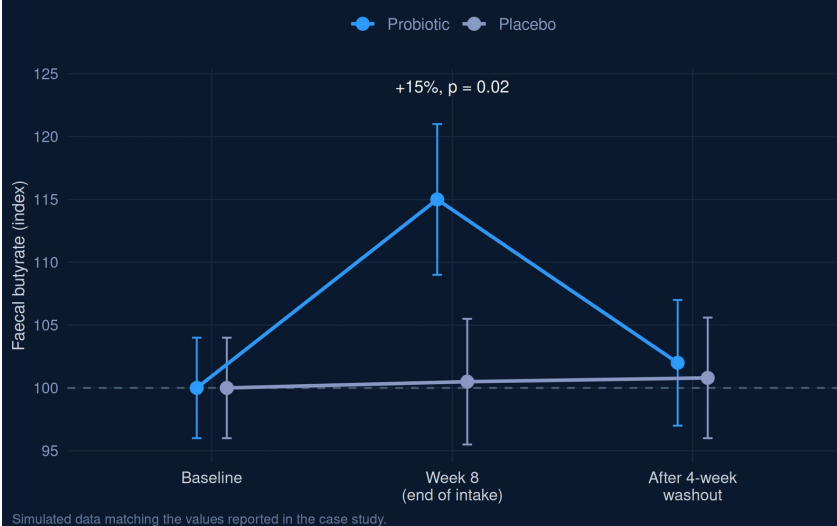
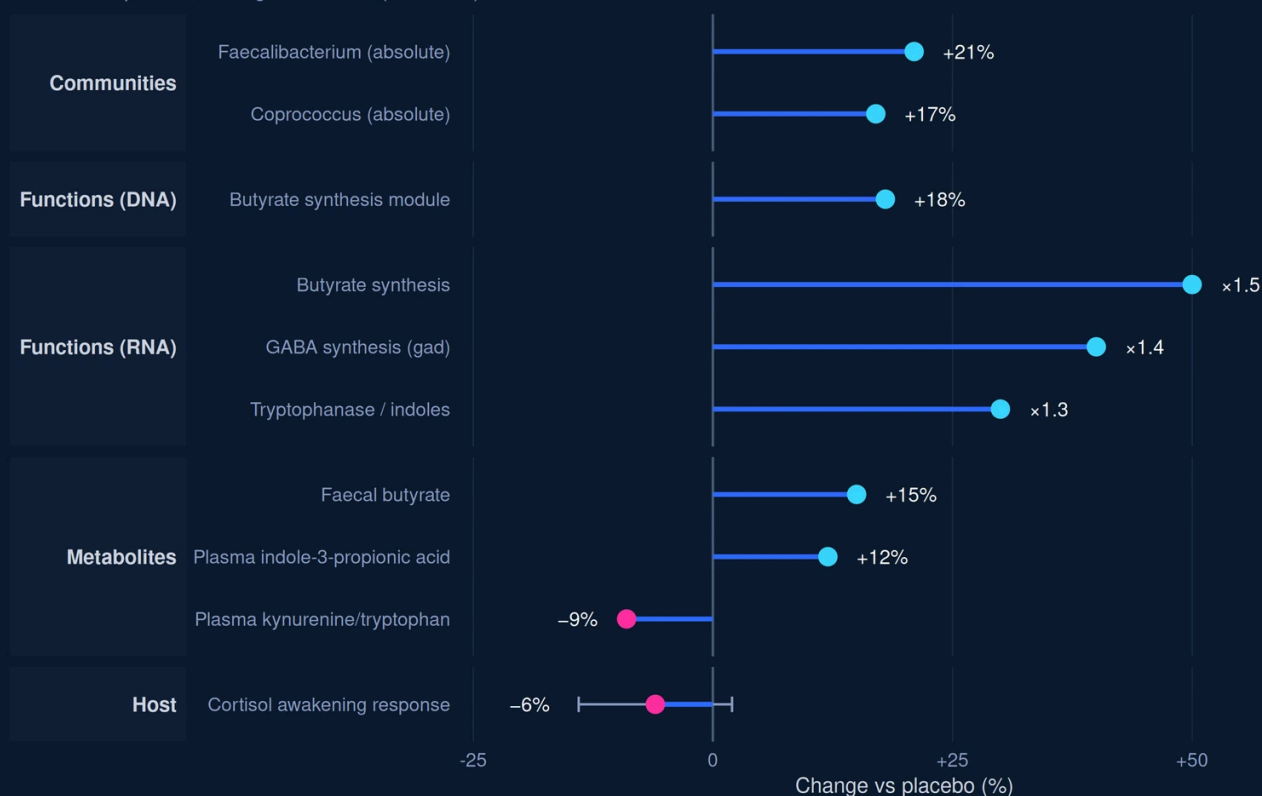


Figure 5. Faecal butyrate over the trial. Mean and 95% CI, indexed to 100 at baseline. In the probiotic arm, butyrate rises by 15% at the end of intake ($p = 0.02$) and returns towards baseline after the 4-week washout, in line with transient engraftment; under placebo it stays flat. Simulated data matching the case study.

What changed, layer by layer

Probiotic vs placebo, change at week 8 (indicative)



Indicative results. Bar = 95% CI where reported. CRP, IL-6 and LBP did not change.

Figure 6. What changed, layer by layer. Change versus placebo at week 8 for each readout, grouped from microbes to host: butyrate producers (absolute abundance), butyrate synthesis genes (DNA), expressed butyrate, GABA and indole pathways (RNA, fold change), metabolites reaching the circulation, and the cortisol response. Points to the right of zero increased, to the left decreased. The cortisol bar is a 95% CI that crosses zero: no effect is shown. Indicative results.

What the client received

- An **integrated dataset**: taxonomic, strain, functional, metabolite and host-marker tables linked by participant and timepoint, with raw data (FASTQ, MS files).
- A **strain-tracking report** documenting the engraftment and persistence of each product strain.
- A **mechanism map** showing which links of the gut–brain chain are supported, partial or open.
- A **statistical report** following the pre-specified analysis plan, including the exploratory mediation.
- **Recommendations for the confirmatory trial**: a higher dose or longer intake to test host signalling; stratification by baseline community to account for colonisation resistance; butyrate and IPA as pre-specified mechanistic endpoints.

INDICATIVE TIMELINE (WEEKS COUNTED FROM THE LAST SAMPLE)

Before the trial	Sampling design, collection kits, SOPs, statistical analysis plan
During the trial	Samples collected at 4 visits, stabilised and biobanked at –80 °C
Weeks 0–6	Extraction, sequencing (shotgun, metatranscriptomics, 16S), metabolomics, host markers
Weeks 4–10	Taxonomy, strain tracking, functional profiling, metabolite annotation
Weeks 8–14	Host-response integration, mediation analysis, final report

Why it works

- **Sampling is half the result.** Stabilisation, standardised collection and controls decide what the data can show.
- **Strains, not just species.** Strain-level tracking proves the product is there, and for how long.
- **Potential, activity, product.** Genes, transcripts and metabolites together show whether a function is present, switched on and reaching the host.
- **Follow the chain to the host.** Each link is tested, not assumed.
- **Name the missing links.** Showing what did not move is what makes the next trial better designed.

Beyond this example

The same approach applies wherever microbiota and health meet:

- **Cardiometabolic health and obesity**: TMAO, bile acids, short-chain fatty acids and energy harvest^{4,3}; responses to diets, fibres, probiotics and drugs.
- **Brain health**: stress, mood and sleep, and neurodegenerative conditions such as Parkinson's⁵ and Alzheimer's disease, where gut and oral microbiota are active research fields.

- **Women's health:** vaginal community state types⁹ across the life course, pregnancy, menopause, intimate-care products and probiotics.
- **Skin and oral health:** cosmetic and oral-care products, inflammatory skin conditions, periodontal health.
- **Study designs:** randomised trials, observational cohorts, case–control studies, in vitro gut models and gnotobiotic animal studies.

References

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Planning a microbiome study?

Tell us your intervention, your matrices and the question behind them. We will propose a design, sampling, sequencing depth, layers and host read-outs, before the first sample is collected. genxmap.com/contact