

1 **TITLE: High-throughput isolation and culture of human gut bacteria with droplet**
2 **microfluidics**

3 **AUTHORS:** Max M Villa^{†‡^}, Rachael J Bloom^{*‡^}, Justin D Silverman^{§||‡}, Heather K
4 Durand^{†‡}, Sharon Jiang^{†‡}, Anchi Wu[°], Shuqiang Huang^{‡°**}, Lingchong You^{‡°}, and
5 Lawrence A David^{†‡§°}

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7 **AUTHOR AFFILIATIONS:**

8 *University Program in Genetics and Genomics, Duke University, Durham, NC, 27708
9 † Department of Molecular Genetics and Microbiology, Duke University, Durham, NC
10 27708
11 ‡ Center for Genomic and Computational Biology, Duke University, Durham, NC 27708
12 § Program in Computational Biology and Bioinformatics, Duke University, Durham, NC
13 27708
14 || Medical Scientist Training Program, Duke University, Durham, NC 27708
15 ° Department of Biomedical Engineering, Duke University, Durham, NC 27708
16 **Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese
17 Academy of Sciences, Shenzhen 518055, People's Republic of China

18

19

20 ^ denotes equal first-authorship

21

22 **CORRESPONDING AUTHOR:**

23

24 Lawrence A David
25 CIEMAS, Room 2171
26 101 Science Drive, Box 3382
27 Durham, NC 27708
28 919-668-5388
29 lawrence.david@duke.edu

30 **Abstract**

31 Isolation and culture of gut bacteria enable testing for microbial roles in disease and
32 may also lead to novel therapeutics. However, the diversity of human gut microbial
33 communities (microbiota) impedes comprehensive experimental studies of individual
34 bacterial taxa. Here, we combine advances in droplet microfluidics and high-throughput
35 DNA sequencing to develop a platform for isolating and assaying microbiota members
36 in picoliter droplets (MicDrop). MicDrop can be used to create millions of distinct
37 bacterial colonies in a single experiment while using off-the-shelf parts compact enough
38 to fit in an anaerobic chamber. In proof-of-concept experiments, we used the platform to
39 characterize inter-individual metabolic variation among hundreds of polysaccharide-
40 degrading gut bacteria from nine stool donors. We also used MicDrop to test the
41 hypothesis that growth kinetics of individual gut bacterial taxa are associated with long-
42 term community dynamics in an artificial gut. These demonstrations suggest the
43 MicDrop platform could support future diagnostic efforts to personalize microbiota-
44 directed therapies, as well as to provide comprehensive new insights into the ecology of
45 human gut microbiota.

46 **Introduction**

47 Bacterial culture was among the first techniques used to study human gut microbiota¹.
48 Bacterial isolation efforts beginning in the early 1900s identified key enteric genera such
49 as *Bacteroides*, *Bifidobacterium*, and *Bacillus*². Microbes isolated since then have
50 served as crucial reagents for experiments. Gut bacterial isolates allow testing causal
51 roles for specific microbes in animal models of metabolic and auto-immune disorders³⁻⁵.
52 Bacterial isolates can also be genetically modified and tested *in vitro* to identify
53 enzymatic machinery in processes like the fermentation of dietary fiber⁶, and cocktails of
54 cultured bacteria are being explored as therapeutics for *C. difficile* infections and
55 cancer⁷⁻⁹.

56 Yet a key challenge for current microbiota culturing efforts has been keeping
57 pace with increasing knowledge and interest in gut microbial diversity. Culture-
58 independent methods based on high-throughput 16S rRNA sequencing have revealed
59 the average individual harbors hundreds of distinct enteric bacterial strains¹⁰⁻¹³.
60 Moreover, unrelated individuals likely share no more than ~30% of bacterial strains¹⁴.
61 Prevailing culture techniques do not scale to the diversity of microbes spanning human
62 populations. Because most taxa are rare, exhaustive capture of bacterial species from
63 even a single stool sample requires laborious spotting of thousands of bacterial
64 colonies^{15,16}. To reduce the human effort needed for such experiments, state-of-the-art
65 culture assays leverage plate and liquid handling robots; but, even these automated
66 efforts tend to be limited to tens of strains^{17,18}. This limitation stems in part from the
67 physical constraints of typical plate-based culture methods, which grow bacteria in wells
68 ranging from centimeters to millimeters in diameter. Even relying on 96- and 384-well

69 plates, conventional large-scale culture efforts may require loading and handling dozens
70 of plates under anaerobic conditions¹⁸.

71 An alternative approach is to culture bacteria in small volumes (nano- to pico-
72 liters) by separating microbes into microscale wells. Devices composed of thousands of
73 such wells have been used to culture both lab strains of bacteria and fungi¹⁹, as well as
74 isolate previously uncultured bacteria from the gut and soil^{20,21}. Even higher-throughput
75 experiments are possible by compartmentalizing microbes in droplets of media that are
76 tens to hundreds of microns in diameter and separated by immiscible oils and
77 engineered surfactants^{22,23}. Because droplets are not limited by the need to
78 microfabricate physical wells or channels, millions of distinct culture volumes can be
79 created on the order of minutes. Droplet techniques have so far been used to isolate
80 uncultured microbes from seawater and soil communities^{20,24,25}, assess microbial cross-
81 feeding²⁶, track population dynamics of individual bacteria²⁷, and examine antibiotic
82 sensitivity and commensal-pathogen interactions of human gut and oral microbiota^{28,29}.
83 Still, existing droplet microfluidic approaches for assaying bacteria have required
84 combining complex emulsion techniques (water-oil-water) with flow cytometers or
85 custom on-chip droplet sorting devices. These protocol requirements limit the
86 accessibility of droplet technologies for bacterial assays and in their present form
87 require equipment that does not fit into typical anaerobic chambers, which are needed
88 to culture human gut bacteria³⁰.

89 Here, we developed a platform to isolate and culture bacteria from human gut
90 microbiota in droplets (MicDrop) using accessible techniques and equipment. A key
91 challenge our method addresses is how to measure the growth of isolates within distinct

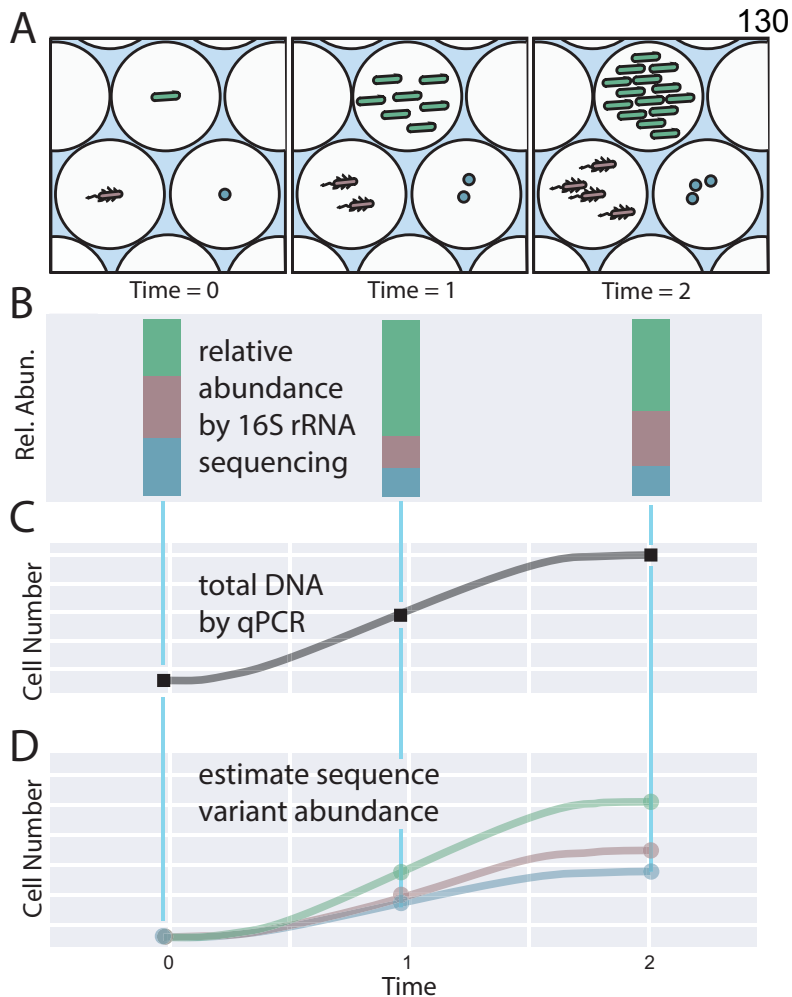
92 microfluidic droplets. To accomplish this, we rely on 16S rRNA as intrinsic DNA
93 barcodes that are shared between droplets carrying the same bacterial taxa, which we
94 refer to here as a sequence variant or SV³¹. This approach in turn allows us to measure
95 isolate growth in droplets without the need for double-emulsion techniques or droplet
96 sorting. Instead, we combine single-emulsion (water-in-oil) microfluidic droplet protocols
97 with molecular techniques (qPCR and 16S rRNA sequencing). These simplified
98 protocols allow us to employ off-the-shelf microfluidic pumps and chips, which are
99 compact enough to fit within typical anaerobic chambers. Using MicDrop, we
100 characterized dietary polysaccharide metabolism among hundreds of gut bacteria from
101 nine individuals. We then employed MicDrop to generate growth curves for dozens of
102 distinct SVs in a single experiment, which we in turn used to investigate long-term
103 microbiota dynamics of an artificial human gut. Together, these findings showcase the
104 potential for microfluidic droplet techniques to characterize the growth and function of
105 individual bacterial strains from complex gut microbial communities in high-throughput.
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108 **Results**

109 **MicDrop: a platform for culturing human gut microbiota in droplets**

110 To isolate and culture individual gut bacteria from human gut microbiota, we
111 merged concepts from prior microfluidic droplet protocols with high-throughput DNA
112 sequencing (Fig. 1; Experimental Procedures). Our protocol first randomly encapsulates
113 individual bacterial cells from gut microbiota into picoliter-sized droplets (Fig. 1A). Gut
114 microbiota samples are diluted before encapsulation using the Poisson distribution at a
115 loading concentration that optimizes the number of droplets loaded with cells (~10-26%)
116 against the number of droplets loaded with more than one microbe (~95-86% of loaded
117 droplets contain single cells) (Supplementary Fig. 1)³². Since many gut bacteria are
118 obligate anaerobes, encapsulation takes place in an anaerobic chamber and droplets
119 are subsequently incubated under anaerobic conditions (Supplementary Fig. 2). To
120 track SV growth, we can avoid having to identify and sort bacteria by assuming that
121 droplets are either empty or loaded with clonal isolates whose progeny share the same
122 16S ribosomal rRNA (rRNA) sequence, meaning genomic material accumulating across
123 all droplets reflects the growth of SVs grown in isolation (Fig. 1A-D). We therefore track
124 isolate growth in droplets at a given time point using bulk bacterial DNA extraction
125 without droplet sorting, followed by DNA sequencing and total quantification (qPCR) of
126 16S rRNA. The product of relative SV levels from 16S rRNA sequencing and total 16S
127 rRNA levels yields an estimate of the absolute levels of each SV across all droplets at
128 the time of sampling.

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131
132 **Figure 1. MicDrop concept** (A) Schematic of bacterial loading and growth in droplets
133 over time. Bulk DNA is collected across all droplets and (B) the 16S rRNA gene is
134 sequenced to establish the relative abundance of each sequence variant. (C) qPCR is
135 used on the same samples to estimate the total abundance of all bacteria. Relative
136 abundances are then multiplied by total bacterial levels to estimate sequence variant
137 abundances (D).
138
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140 To explore the feasibility of the MicDrop platform, we initially examined bacterial
141 replication and isolation in droplets, as well as the use of DNA sequencing to track
142 bacterial levels. We found aerobic monocultures of fluorescent *Escherichia coli*
143 replicated in droplets in a qualitative manner that resembled growth on conventional
144 Petri dishes (Supplementary Fig. 3). Droplet stability experiments suggested that

145 bacteria could be studied in droplets for at least 5 days (Supplementary Fig. 4). Next,
146 microscopy showed droplets could be used to segregate clonal isolate populations with
147 distinct morphology and motility out of mixed microbial communities (Supplementary
148 Fig. 5). Human fecal microbiota isolated and cultured in droplets exhibited 2.6 times
149 more diversity than when grown in mixed conditions (Supplementary Fig. 6), which is
150 consistent with the hypothesis that droplet isolation enables slow-growing microbes to
151 be sheltered from competition with fast-growing bacteria²⁰. Last, we found DNA-based
152 techniques could be used to track bacterial levels in droplets. Quantitative growth
153 measurements using qPCR of the 16S rRNA gene sampled every two hours from liquid
154 cultures were also similar to *E. coli* grown on plates ($\rho=0.95$, $p=8.7e-9$; Spearman
155 correlation; Supplementary Fig. 7). Next, using DNA sequencing, we analyzed a mixture
156 of 10 bacterial strains that were isolated using MicDrop and grown under varying
157 antibiotic conditions for 24 hours. We found that resulting bacterial DNA levels in
158 droplets corresponded to isolates' optical densities in reference well-plates
159 (accuracy=75%; Supplementary Fig. 8).

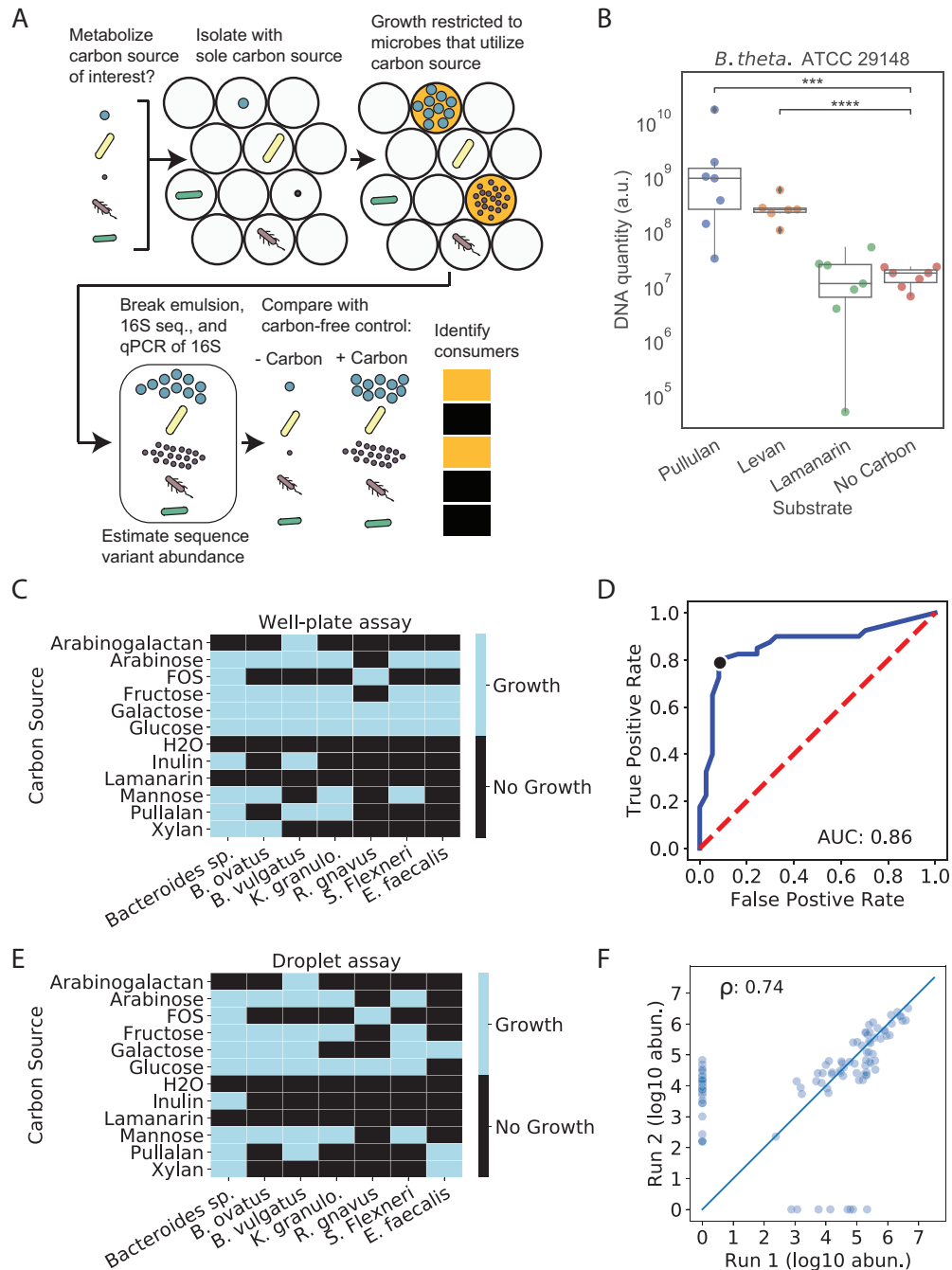
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161 **A droplet assay for prebiotic consumption by human gut microbes**

162 To demonstrate how MicDrop could be applied to problems in human gut microbiology,
163 we used the platform to measure bacterial utilization of carbohydrates. In typical
164 carbohydrate utilization screens, bacteria are cultured in defined media containing a
165 carbohydrate as the sole carbon source^{17,33}. Microbes that replicate are assumed to be
166 capable of utilizing the carbohydrate and are termed "primary degraders"^{34,35}. The
167 biology of primary degraders is of increasing interest because bacterial metabolism of

168 select indigestible carbohydrates (prebiotics) leads to the growth and activity of gut
169 microbes with multiple beneficial impacts on host health^{17,36-40}. Still, bacterial prebiotic
170 metabolism is incompletely understood, particularly with regards to the origins of wide
171 inter-individual variation in microbiota metabolic potential⁴¹⁻⁴³.

172 To explore how the MicDrop platform could assay bacterial prebiotic metabolism
173 (Fig. 2A), we first loaded a previously characterized type strain *Bacteroides*
174 *thetaiotaomicron* ATCC 29148 into microfluidic droplets and standard 96-well plates.
175 Consistent with both prior studies¹⁷ and our well-plate experiments, *B. thetaiotaomicron*
176 ATCC 29148 replicated in droplets on pullulan and levan, but not on laminarin or a no
177 carbohydrate control (Fig. 2B). We next tested how the MicDrop prebiotic assay
178 performed using artificial microbial communities assembled from seven human gut
179 isolates (Fig. 2E). Using 96-well plate experiments as our reference (Fig. 2C & 2D,
180 Supplementary Fig. 9), we found the sensitivity, specificity, and false discovery rate of
181 the MicDrop prebiotic assay to be 80%, 93%, and 9%, respectively (Supplementary
182 Table 1). Finally, to assess the reproducibility of the MicDrop prebiotic assay, we used
183 the same frozen fecal sample to compare the results of two separate experimental
184 sessions. We observed higher correlation between replicates from the same session
185 ($\rho = 0.73-0.78$, $p < 0.0001$, Spearman correlation) than between replicates across
186 sessions ($\rho = 0.57$, $p = 1.67e-17$, Spearman correlation). One explanation for the
187 difference in correlation is that microbial communities re-assembled in different
188 configurations each time microbiota was revived from frozen stool⁴⁴. Indeed, controlling
189 for microbiota differences between droplet inocula elevated the between session
190 correlation ($\rho = 0.74$, $p = 5.14e-19$, Spearman correlation, Fig. 2F).



191
192 **Figure 2. A prebiotic utilization screen based on the MicDrop platform**
193 (A) Schematic of MicDrop prebiotic assay. (B) Droplet monoculture growth of *B.*
194 *thetaiotaomicron* in microfluidic droplets measured by qPCR. (C) Results of 96-well
195 plate growth of gut bacterial isolates across 11 carbohydrates. (D) ROC curve of
196 MicDrop assay results at different growth threshold cut-offs using (C) as a reference.
197 The black dot indicates the growth threshold that maximizes the true positive rate while
198 minimizing the false positive rate, depicted in (E). (F) Correlation between two different
199 MicDrop sessions (each carried out in triplicate) on the same frozen fecal sample and
200 five different carbohydrates. Points indicate median growth of different SVs across each
201 experimental session.

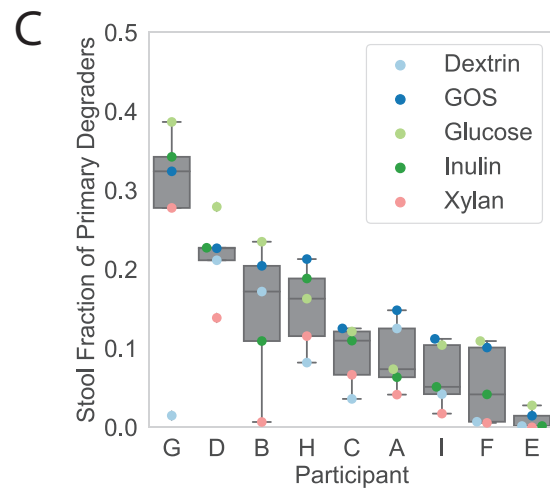
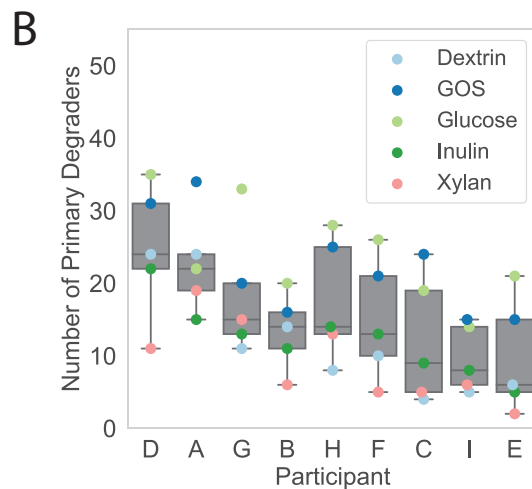
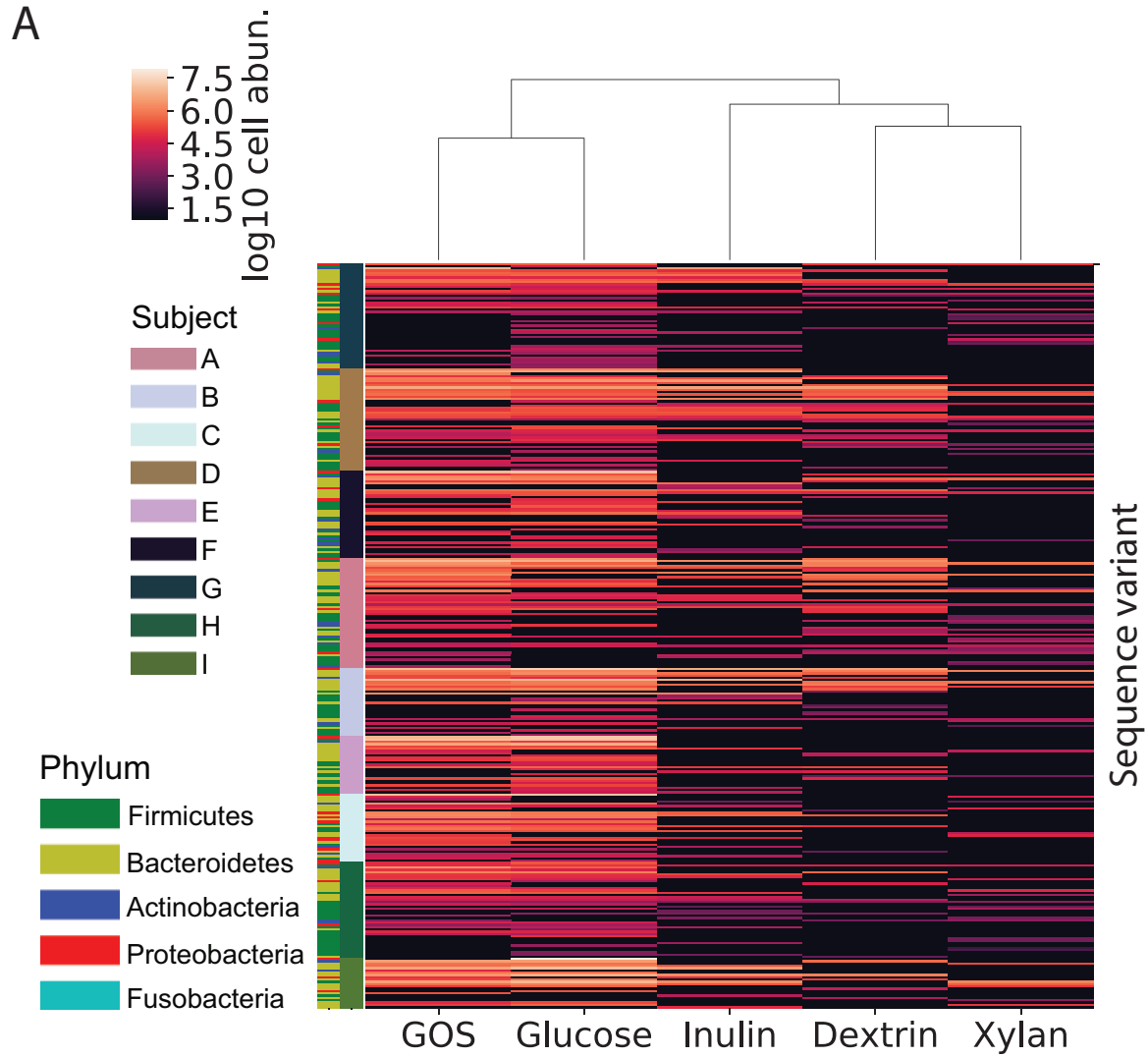
202 **Identifying primary degraders from human guts across multiple prebiotics**

203 We next applied the MicDrop prebiotic assay to microbiota from nine healthy
204 human stool donors. We assayed growth on three consumer-grade prebiotics (inulin,
205 galacto-oligosaccharides (GOS), and dextrin) and a lab-grade prebiotic (xylan). Out of
206 the 588 SVs detected across donor stool samples, MicDrop identified a total of 285
207 primary degraders that grew on at least one of the screened prebiotics (Fig. 3A).
208 Prebiotic utilization patterns of primary degraders were similar to those of matched
209 bacterial strains in a reference database of microbial carbohydrate utilization^{45,46}.
210 Among the instances when matched bacteria were annotated as consuming a prebiotic
211 in the database, 86% were detected by MicDrop ($p < 0.001$; permutation test). Still, we
212 did find that 52% of instances when MicDrop indicated a primary degrader to consume
213 a prebiotic were not reported for matched bacteria in the database ($p = 0.34$; permutation
214 test), which may in part reflect incomplete enumeration of bacterial carbohydrate
215 utilization in the reference.

216 We next explored the hypothesis that differences in the presence or absence of
217 primary degraders could drive inter-individual variation in human prebiotic response.
218 Evidence arguing against this hypothesis included observing that multiple SVs capable
219 of growing on the tested prebiotics were present in all subjects (median: 12.5 ± 6.1);
220 Fig. 3B, Supplementary Table 2). Additionally, primary degraders were more likely to be
221 shared between individuals than SVs not identified as primary degraders in stool
222 samples ($p = 0.00122$, Chi-square test). Concordant with human studies showing that
223 even individuals with low prebiotic fermentation *in vivo* exhibit at least some prebiotic

224 fermentative capacity *in vitro*³⁵, our findings support the hypothesis that primary
225 degraders are found across most individuals.

226 Still, we also found evidence for inter-individual variation in primary degrader
227 composition and abundance. We observed differences in the richness of primary
228 degraders across subjects ($p < 0.001$, Two-way ANOVA; Fig. 3B). We also found subject
229 identity explained more variation ($R^2 = 0.30$, PERMANOVA; Supplementary Table 3) than
230 prebiotic type ($R^2 = 0.16$, PERMANOVA; Supplementary Table 3) in overall primary
231 degrader growth. Last, we observed differences in the relative abundance of primary
232 degraders in inoculating fecal communities ($p < 0.0001$, Two-way ANOVA; Fig. 3C).
233 Thus, while primary degraders are likely present in most individuals, differences in
234 polysaccharide metabolism may be due to inter-individual variation in primary degrader
235 abundance in the gut^{41,42}.



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Figure 3. MicDrop prebiotic assay carried out on fecal samples from nine individuals. (A) Microbial carbohydrate preferences for 328 SVs from nine healthy human donors. Of these SVs, 285 grew on at least one of the prebiotics (*i.e.* GOS,

240 inulin, dextrin, or xylan). (B) The number of primary degraders detected by MicDrop and
241 (C) the relative abundance of primary degraders in stool samples differed by participant
242 and prebiotic ($p < 0.001$, Two-way ANOVA). Participant orderings in (B) and (C) are
243 sorted by median values.

244
245

246 **High-throughput estimation of growth dynamics of human gut microbiota**

247 In a second set of demonstration experiments, we used the MicDrop platform to
248 explore the dynamics of human gut microbiota in artificial gut systems. Such systems
249 have been used when *in vivo* microbiota research is challenging, including measuring
250 the effects of nutrition on the infant gut, systematic antibiotic testing, and investigating
251 chemotherapy-induced dysbioses^{47,48,49}. Still, a recurring challenge of artificial gut
252 models has been their inability to completely reconstruct *in vivo* microbial communities.
253 After inoculation, these models often decrease in diversity within 24 hours⁵⁰⁻⁵², and as
254 little as 15% of the starting community may ultimately remain after a week of culture⁵³. A
255 potential explanation for part of this diversity loss is that bacteria are sensitive to media
256 conditions: studies using individual gut bacterial strains show growth can be affected by
257 even a single medium component⁵⁴; and, varying media used in artificial guts leads to
258 broad scale changes in microbial community structure^{55,56}. Still, the hypothesis that
259 individual gut microbes' suitability to media is associated with their long-term
260 persistence in artificial gut models has not been fully explored, likely due in part to the
261 challenges of isolating and assaying each component species in these communities.

262 To test the hypothesis that growth of individual microbial SVs in a particular
263 medium would correspond to SV persistence in an artificial gut setting, we used stool
264 from a healthy human donor to inoculate a continuous flow bioreactor system we have
265 used in past gut microbial ecology studies^{57,58}. The artificial gut was supplied with

266 modified Gifu Anaerobic Medium (mGAM)⁵⁹, which features a variety of carbon and
267 nitrogen sources, as well as extra amino acids, vitamin K, and hemin. We chose mGAM
268 because it enables a wide growth of mammalian gut bacteria^{54,59}. Yet, despite this
269 choice of medium, microbiota dynamics in the artificial gut exhibited the same loss in
270 diversity observed in prior studies (Supplementary Fig. 10)^{50,53}. At the end of two weeks
271 of culture, only 23% and 18% of inoculating bacteria genera and SVs, respectively,
272 were still detected in the artificial gut (Supplementary Table 4).

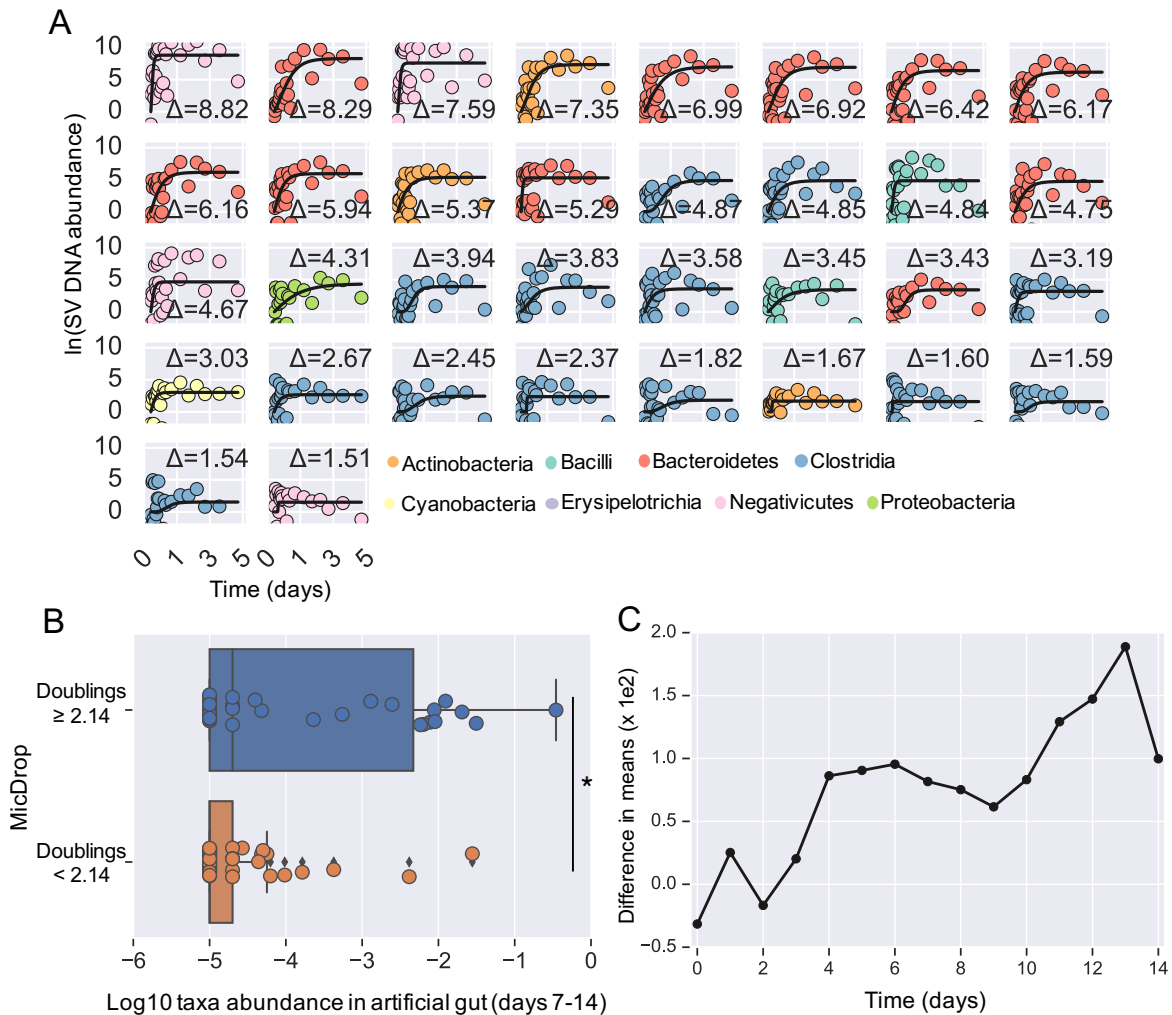
273 A fresh stool sample from the same donor used to inoculate the artificial gut was
274 then assayed by the MicDrop platform. To additionally demonstrate the potential for
275 MicDrop to assess the kinetics of bacterial growth over time, we created replicate
276 droplet populations and destructively sampled them at hourly intervals for the first 24
277 hours, and daily for four subsequent days after inoculation. Among the resulting time
278 series, 94 SVs were detectable in droplets, meaning they appeared in >5 time points;
279 Table 1, Supplementary Fig. 11). These SVs included representatives from the major
280 human gut bacterial phyla (the Actinobacteria, Bacteroidetes, Firmicutes, and
281 Proteobacteria) and represented 76% of the inoculum's SVs, a proportion approaching
282 prior culture efforts using mGAM medium⁵⁹. Of the detectable SVs, we then measured
283 how many exhibited evidence for growth in droplets. We defined a cut-off for growth as
284 inferred doublings of at least 2.14 times ($\Delta \ln(\text{SV DNA abundance}) \geq 1.48$) based on our
285 antibiotic-based control experiments (Supplementary Fig. 8). A total of 34 SVs were
286 defined as growing (Fig. 4A), which accounted for 25% of the inoculum's SVs. Of the
287 SVs with positive growth, 12 SVs were not detected by sequencing in the inoculum,
288 which suggests they could be laboratory contaminants. Still, these SVs resemble known

289 gut bacteria (Supplementary Table 5) and may alternatively represent rare microbes
290 that require culture to be detected, which is a previously reported phenomenon⁶⁰.

291 **Table 1.** Number and fraction of microbes from a human stool sample cultured by
292 MicDrop in mGAM medium. SVs were considered as ‘detected’ if present in more than
293 five longitudinal measurement. ‘Growth’ was defined by an inferred number of doublings
294 equal or greater than 2.14.
295

Taxonomic level	Taxa in inoculum	Taxa detected in droplets	Taxa in inoculum & detected in droplets	Fraction of inoculum detected in droplets	Taxa grew in droplets	Taxa in inoculum & grew in droplets	Fraction of inoculum that grew in droplets
Phylum	4	5	4	1.00	5	4	1.00
Class	10	11	10	1.00	7	5	0.50
Order	10	14	10	1.00	8	5	0.50
Family	17	21	16	0.94	13	7	0.41
Genus	56	53	40	0.71	20	13	0.23
Sequence Variant	89	94	68	0.76	34	22	0.25

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299 **Figure 4. Comparison of SV growth kinetics and persistence in an artificial gut.**

300 (A) Abundance over time of SVs in MicDrop from a fresh human fecal sample. Modified

301 Gompertz growth curves are fit to time-series. SVs are colored by taxonomy and sorted

302 according to total growth (curve asymptote height; indicated by Δ), which is denoted on

303 each sub-plot. Only SVs inferred to double at least 2.14 times were considering growing and

304 are shown ($\ln(\Delta \text{ SV DNA abundance}) \geq 1.48$; threshold determined using control

305 experiments in Supplementary Fig. 8). To ease viewing, curves are shifted vertically so

306 y-intercepts are at the origin. (B) Long-term abundance of SVs in an artificial gut

307 (grouped across days 7-14) grouped by whether SVs were identified by MicDrop as

308 growing (doubling ≥ 2.14 times) or non-growing doubling < 2.14 times) (Mann-Whitney

309 U, $p < 0.02$). (C) Differences in mean abundances of growing and non-growing SVs

310 increased over time in an artificial gut system ($\rho = 0.80$, $p < 0.0004$, Spearman

311 correlation).

312

313 The growth of SVs measured with MicDrop were ultimately associated with SV
314 dynamics in the artificial gut. Such associations were not apparent on short-time scales
315 (i.e. 1-5 days after inoculation), which is consistent with the notion that non-growing SVs
316 require several days to wash out of an artificial gut after inoculation. However, from day
317 7-14 of the artificial gut experiment, we observed elevated abundances among artificial
318 gut SVs that grew in the MicDrop platform (inferred doublings ≥ 2.14) relative to ones
319 that did not (SV doublings < 2.14) ($p < 0.02$, Mann-Whitney U test) (Fig. 4B). This
320 difference in abundance increased over time in the artificial gut system ($\rho = 0.80$, $p <$
321 $1e-4$, Spearman correlation) (Fig. 4C). Still, some SVs grew well in droplets, but did not
322 persist in the artificial gut (left-most points of upper bar in Fig. 4B); or, by contrast, did
323 not grow in droplets, but were relatively abundant in the artificial gut (right-most points of
324 lower bar in Fig. 4B). The former may represent examples of SVs that are outcompeted
325 in mixed culture, while the latter may be examples of SVs that depend on inter-species
326 interactions to persist.

327

328 **Discussion**

329 We report here a microfluidic platform for isolating, culturing, and assaying
330 component members of human gut microbiota (MicDrop) using accessible microfluidic
331 and molecular techniques. We used MicDrop to compare the growth kinetics of dozens
332 of microbial SVs to the dynamics of an artificial gut community and to examine inter-
333 individual variation in gut bacterial polysaccharide metabolism. The flexibility of the
334 platform suggests its underlying concepts could be applied to assaying microbial
335 responses to other compounds including pharmaceuticals, antibiotics, or host-secreted

336 compounds^{18,61} using individual members of communities comprised of microbes from
337 culture collections, mutant libraries, other human body sites, or environmental systems.
338 The ability of MicDrop to screen clonal populations could be particularly useful for
339 assays characterizing the behavior of isolates free from the effects of inter-species
340 interactions like competition or facilitation^{17,18}.

341 Yet, we acknowledge MicDrop still has some limitations. We rely on 16S rRNA as
342 a molecular barcode for droplets sharing the same bacterial SV, meaning that the
343 platform is sensitive to similar challenges due to inter-species rRNA copy number
344 variation confronting 16S rRNA microbiota surveys⁶²; and MicDrop cannot detect
345 differences in growth originating from distinct clones of the same SV. For precise growth
346 assays targeting bacteria from a limited number of taxa, traditional culture methods
347 could be better suited. An additional limitation of MicDrop in its current form is the time
348 and manual effort needed to setup individual droplet generation experiments and
349 ensure accurate Poisson dilution of bacterial cells. Experimental effort could be reduced
350 and reproducibility enhanced by automating sample switching. Last, we focused here on
351 culture in liquid media using soluble substrates; future extensions of MicDrop that
352 provide solid physical surfaces to colonize^{63,64} or insoluble substrates like mucin will
353 require developing techniques to avoid the clogging of microfluidic channels.

354 Still, in its present form, MicDrop enabled useful insights into human gut
355 microbiology. Our findings that bacterial SV growth in isolation is associated with
356 persistence in an artificial gut supports the ecological hypotheses that intrinsic lifestyle
357 characteristics of bacteria shape overall community dynamics. Indeed, species' growth
358 rate (measured by 16S rRNA copy number) is positively correlated with microbes'

359 relative abundance in seawater⁶⁵, as well as skin microbiota of amphibians⁶⁶.
360 Additionally, our microfluidic investigation of prebiotic response supports hypotheses
361 that inter-individual variation to carbohydrate interventions is due to differential
362 abundances of polysaccharide degrading bacteria between people^{43,67}. Droplet
363 microfluidics could be used in the future to stratify human populations into groups most
364 likely to benefit from prebiotic treatments⁴¹, by providing a culture-based diagnostic
365 approach capable of scaling to the diversity of microbes inhabiting the human gut.
366

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581

582 **EXPERIMENTAL PROCEDURES**

583

584 **Overall MicDrop procedure**

585 Droplets were made on a microfluidic chip (6-junction droplet chip, Dolomite
586 Microfluidics). Bacterial media varied by assay; for the oil phase, we used a fluorinated
587 oil and surfactant mixture 1% Picosurf (Sphere Fluidics) in Novec 7500 (3M). One day

588 prior to performing the droplet assay, all reagents including carrier oil, culture media,
589 and carbon solutions were equilibrated to the anaerobic atmosphere in an anaerobic
590 chamber (Coy). The fecal inoculum optical density at 600 nm was recorded and diluted
591 according to the Poisson distribution: $P(n, \bar{n}) = \frac{\bar{n}^n e^{-\bar{n}}}{n!}$, where n is the droplet occupancy
592 (i.e. 0, 1, .. cells/droplet) and \bar{n} is the average number of cells per droplet given by: $\bar{n} =$
593 ρV , where V is droplet volume and ρ is cell density. Assays were performed at a \bar{n} of
594 0.1-0.3 to minimize the number of droplets loaded with more than one cell
595 (Supplementary Fig. 1). Thus, for a fixed droplet volume and \bar{n} , the target cell
596 concentration can be obtained from: $\rho = K \frac{\bar{n}}{V}$, where K is a constant that converts
597 CFUs/mL to OD₆₀₀ determined from replicate CFU assays. Syringe pumps were used
598 to control the flow rates of the oil and cell suspension (NE-1000 Single Syringe Pump,
599 New Era Pump Systems). Following the culture period, droplets were loaded into
600 chambered slides (C10283, Invitrogen) or directly onto glass slides and observed with
601 Phase and/or Darkfield microscopy (Nikon) to examine growth and the appropriate
602 loading. All steps of cell encapsulation and culture were performed in an anaerobic
603 chamber.

604

605 **Collection and preparation of fecal inoculum for artificial gut and MicDrop growth** 606 **dynamics assays**

607 Stool was collected from human donors under a protocol approved by the Duke
608 Health Institutional Review Board (Duke Health IRB Pro00049498). Inclusion criteria
609 limited this study to healthy subjects who could provide fecal samples at no risk to
610 themselves, had no acute enteric illness (e.g. diarrhea) and had not taken antibiotics in

611 the past month. Fresh stool samples were collected in a disposable commode specimen
612 container (Fisher Scientific, Hampton NH). Intact stool was moved within roughly 15
613 minutes of bowel movement into anaerobic conditions. The sample was prepared for
614 inoculation in an anaerobic chamber (Coy). A 5 g stool aliquot was weighed into a 7 oz
615 filtration bag (Nasco Whirl-Pak) and combined with 50 mL of mGAM media (Gifu
616 Anaerobic Medium, HiMedia, with the addition of 5 mg/L Vitamin K and 10 mg/L Hemin,
617 ⁵⁹) that was pre-reduced overnight in an anaerobic chamber. The mixture was
618 homogenized in a stomacher (Seward Stomacher 80) on normal speed for 1 minute
619 under atmospheric conditions to make a total of 100 mL of inoculum. The supernatant
620 was decanted into beakers and loaded into syringes for inoculation into the artificial gut
621 or filtered through a 50 μ m filter (Celltrics) and diluted and loaded into droplets.

622

623 **Droplet DNA extraction, PCR amplification, and DNA sequencing**

624 To extract DNA from droplets, excess oil was removed by pipetting and water-in-
625 oil emulsions were broken by adding an equal amount of 1H,1H,2H,2H-Perfluoro-1-
626 octanol (PFO, VWR) and briefly vortexed. Then, the samples were briefly centrifuged
627 (<200 g) to separate the aqueous and oil phases by density. The aqueous solution was
628 transferred to a new tube, and DNA was extracted using a kit (Qiagen #12224). DNA
629 was extracted from artificial gut and stool samples using a 96-well PowerSoil kit (Qiagen
630 #12888). For all samples, the V4 region of the 16S rRNA gene was barcoded and
631 amplified from extracted DNA using with custom barcoded primers, using published
632 protocols^{68,69}. 16S rRNA amplicon sequencing was performed on an Illumina MiniSeq
633 with paired-end 150 bp reads. We chose to only analyze samples with more than 5,000

634 reads to remove outlying samples that may have been subject to library preparation or
635 sequencing artifacts. The 16S rRNA nucleotide sequences generated in this study will
636 be made available at the European Nucleotide Archive under study accession number
637 TBD. Total bacterial abundances from droplet cultures were estimated by qPCR for
638 bacterial 16S rRNA using the same primers used in the DNA sequencing protocol.
639 Amplification during the qPCR process was measured with a Real-Time PCR system
640 (CFX96 Real-Time System, BioRad) using *E. coli* DNA at a known cell concentration as
641 a reference.

642

643 **Identifying Sequence Variants and Taxonomy assignment**

644 DADA2 was used to identify SVs³¹. Custom scripts were used to prepare data for
645 denoising with DADA2 as previously described⁵⁷. Reads were then demultiplexed using
646 scripts in Qiime v1.9⁷⁰. SVs were inferred by DADA2 using error profiles learned from a
647 random subset of 40 samples from each sequencing run. Bimeras were removed using
648 the function `removeBimeraDenovo` with `tableMethod` set to “consensus”. Taxonomy was
649 assigned to sequence variants using a Naïve Bayes classifier⁷¹ trained using version
650 123 of the SILVA database⁷². For growth dynamics of the human gut microbiota and
651 microbiota dynamics in the artificial gut, only forward sequencing reads were analyzed.
652 Downstream analysis on sequence variant tables was performed using R (ver. 3.4.2)
653 and Python (ver. 2.7.6). PERMANOVA was run in R using `adonis` in the `vegan` package
654 (ver. 2.5-2).

655

656 **Growth dynamics of human gut microbiota**

657 To estimate SV growth curves using MicDrop, we collected a total of 70 separate
658 microfluidic droplet aliquots for destructive longitudinal sampling. Droplets were
659 generated according to the MicDrop protocol described above. We used a modified Gifu
660 Anaerobic Medium (mGAM) in our droplets (Gifu Anaerobic Medium, HiMedia, with the
661 addition of 5 mg/L Vitamin K and 10 mg/L Hemin). Each aliquot of 200 μ l of droplets
662 was incubated at 37 °C in an anaerobic chamber. Aliquots were destructively sampled
663 in triplicate, hourly, for hours 0-24 after droplet making and in duplicate once a day for
664 hours 24-127 after droplet making.

665 Growth curves were fit using a combination of 16S rRNA qPCR and DNA
666 sequencing data. To minimize the potential for poorly fit growth curves, SVs were
667 required to have been detected by DNA sequencing in >5 samples to be included in
668 curve fitting. To avoid numerical instabilities associated with taking the log or dividing by
669 zero, a pseudocount of one was added to the sequence variant count table prior to
670 normalization to relative abundances. Relative abundances of each SV were then
671 determined by dividing the number of counts associated with each SV in each sample
672 by the total read counts in the sample. Concentrations of each taxa were then estimated
673 by multiplying the relative abundances of SVs by the 16S rRNA concentrations
674 determined by qPCR. Technical replicates constituted distinct data points in these
675 calculations. We used the SciPy Python package (v0.19.1) to fit a modified Gompertz
676 equation⁷³ to which we added an additional term to account for differences in starting
677 abundance to the resulting dataset: $y = A \exp \left\{ -\exp \left[\frac{\mu \cdot e}{A} (\lambda - t) + 1 \right] \right\} + A_0$, where μ is
678 growth rate, A is carrying capacity, λ is lag time, or the time it takes for a bacteria to
679 reach logarithmic growth, and A_0 accounts for the relative abundance of different SVs in

680 the inoculum. We fit curves using the module `scipy.optimize.least_squares` with the
681 robust loss function “`soft_l1`”. Parameter bounds were also used to minimize the
682 optimization search space. We set lower bounds of $A=0$, $\lambda=-50$, $\mu=0$, $A_0=0$; and, upper
683 bounds of $A=15$, $\lambda=12$, $\mu=2.6$, $A_0=15$. We selected bounds by considering both
684 biological feasibility and parameter sensitivity analyses (Supplementary Fig. 12). Our
685 upper bound for growth rate ($\mu=2.6$) represented a doubling time of 15 minutes, which
686 we based on the fastest growth rates observed in an anaerobic bacterium⁷⁴. The upper
687 bounds on A and A_0 were set to the maximum amount of DNA measured across
688 replicate MicDrop samples from the human fecal inoculum. The upper bound on λ ,
689 which represents the lag time until exponential growth⁷⁵ was set at 12. Lower bounds of
690 0 for A , A_0 , and μ reflect our choice not to model negative growth. A lower bound for λ
691 was selected by sensitivity analysis (Supplementary Fig. 12), which revealed that a
692 bound of zero led to fitted λ values regularly collapsing to our boundary limits. We also
693 found that fitted curves were sensitive to starting parameters. To ensure a broad search
694 of parameter space, we initialized each curve fit multiple times ($n=100$) with starting
695 parameters randomly distributed between the bounds of each parameter. Fitted growth
696 rates often collapsed to the maximum μ tolerated; we therefore only retained fits where
697 growth rates were at least slightly below our upper bound for μ ($\mu < 2.5$) (Supplementary
698 Fig. 13). Of the remaining fitted curves, we analyzed the one with the lowest loss
699 function. In our analyses of SV growth in human fecal samples, we defined total SV
700 levels as $y(127 \text{ hours}) - y(0 \text{ hours})$.

701

702 **Microbiota dynamics in an artificial human gut**

703 We cultured human gut microbiota with an artificial gut model that we have used in prior
704 studies^{57,76}. A continuous-flow artificial gut system (Multifors 2, Infors) was used to
705 culture gut microbiota seeded from human stool. A vessel was autoclave-sterilized and
706 prepared with 300 mL of mGAM media (see *Growth dynamics of human gut microbiota*
707 above). We inoculated the vessel with 100 mL of fecal inoculum, resulting in a total
708 culture volume of 400 mL. After 24 hours, the media feed was initiated at a constant
709 rate of 400 mL per day. A carboy feeding the media was changed once over the course
710 of the 14 days. Feed rate, oxygen, pH, temperature, and stir rate were all controlled by
711 software (IRIS v6, Infors). Positive pressure within the vessels was maintained to
712 prevent contamination by sparging with nitrogen at 1 LPM. Dissolved oxygen
713 concentration was measured continuously using Hamilton VisiFerm DO Arc 225 probes.
714 pH was monitored with Hamilton EasyFerm Plus PH ARC 225 probes and was
715 maintained between 6.9 and 7.1 using a 1 N HCl solution and a 1 N H₃PO₄ solution.
716 The vessel was maintained at 37°C via the Infors' onboard temperature control system.
717 The vessel was continuously stirred at 100 rpm using magnetic impeller stir-shafts.
718 Samples were taken once every 24 hours between 1 PM and 5 PM for 14 days and
719 were frozen immediately at -80°C for later extraction.

720

721 **MicDrop Prebiotic Assay**

722 Microbial communities isolated from human stool samples were tested for carbohydrate
723 consumption using the MicDrop platform. Cells were revived from frozen stock in rich
724 medium (mGAM, see *Growth dynamics of human gut microbiota*) for 18 hours to allow
725 cells to recover from freezing. Bacteria were then cultured in minimal medium

726 (Supplementary Table 6) containing glucose and galactose (Sigma) as the sole carbon
727 sources to deplete excess nutrients⁷⁷. Following determination of the loading
728 concentration, the bacteria were washed twice by centrifugation (2 min at 14,000 g) to
729 remove free monosaccharides and resuspended in 2X minimal medium without a
730 carbon source. Bacteria were filtered using a 50 µm filter (CellTrics, Sysmex) to remove
731 multi-cell clumps. The filtered microbiota suspension was then added to prebiotics in a
732 50:50 mixture of 1% prebiotic solution and 2X minimal medium. To prevent chip fouling
733 during droplet generation, the oil inlet was equipped with 10 µm inline filters (P-276,
734 IDEX). Droplet generation in the anaerobic chamber was monitored using a bright field
735 microscope (Celestron). Droplet cultures were stored in 5 mL polypropylene tubes
736 (Falcon) with the caps closed in an anaerobic incubator at 37 °C. Following the second
737 day of incubation, cultures were moved to a -20 °C freezer for storage prior to DNA
738 extraction.

739

740 **Validation of prebiotic utilization assays**

741 To validate the MicDrop prebiotic assay, we generated reference data on carbohydrate
742 preferences using an artificial community of seven wild-type gut isolates from our
743 culture collection (Supplementary Table 7), which were grown in both 96-well plates and
744 the MicDrop Prebiotic Assay described in the preceding paragraph. Following the same
745 procedure described in the *MicDrop Prebiotic Assay*, well plates were prepared with
746 minimal medium (Supplementary Table 6) and a carbohydrate as a sole carbon source
747 (Supplementary Table 8). A 10 µL aliquot of bacterial suspension was added to 200 µL
748 of medium in 96-well plates and incubated in a humidified container for two days at 37

749 °C. All culture experiments were performed in an anaerobic chamber. Following the
750 culture period, the optical density at 600 nm of each well was examined using a plate
751 reader (CLARIOstar, BMG Labtech). Following published protocols¹⁷, isolate growth in
752 plates was normalized to the maximum growth for each microbe. To classify isolates as
753 either “growing” or “not-growing,” a threshold of 20% of maximum growth was applied to
754 the plate data, above which was considered growth on the carbon source of interest.
755 The same isolates used in the well-plate analyses were mixed evenly into an artificial
756 community and examined using the MicDrop prebiotic assay described above. MicDrop
757 experiments were performed in triplicate. Growth thresholds for the MicDrop assay were
758 determined by first pre-processing sample qPCR values to zero if they indicated overall
759 growth below mean no-carbon controls. Then, relative SV abundance data were
760 converted to absolute SV abundances by multiplying each sample by the corresponding
761 qPCR value. Median SV abundances were then calculated across replicates and SV
762 abundances from matched no-carbon controls were subtracted from each sample. An
763 optimal SV growth threshold for determining growth on a carbohydrate in MicDrop was
764 determined by applying Youden’s J index across all possible threshold values, with the
765 well-plate data as the reference (Supplementary Fig. 9). A growth threshold of 88%
766 maximized this index and was used in subsequent experiments on fecal samples.
767

768 **MicDrop prebiotic assays using human stool samples**

769 Stool samples were collected from nine healthy donors (7 men, 2 women) between the
770 ages of 35-53 under the IRB protocol described in section *Collection and preparation of*
771 *fecal inoculum for artificial gut and MicDrop growth dynamics assays*. To facilitate

772 carrying out prebiotic assays simultaneously across a range of donors, we used frozen
773 gut microbiota in these experiments. Fecal slurries were made at 10% w/v using mGAM
774 medium and a stomacher (Seward) that homogenized fecal samples for one minute.
775 Then, slurries were mixed 50:50 with 50% glycerol and stored at -80 °C for later use.
776 Samples were assayed following the MicDrop prebiotic assay procedure described
777 above.

778

779 **Comparison of primary degraders to the Virtual Metabolic Human database**

780 We compared the identity of primary degraders to carbon consumption profiles from the
781 Virtual Metabolic Human (VMH) database^{46,78}. We first mapped primary degraders to
782 this database by taking SV 16S rRNA sequences from our study and searching for
783 matches in the NCBI nucleotide database. Each 100% match was then linked to a type
784 strain in the VMH database using NCBI taxonomy ids. Since some NCBI taxonomy IDs
785 could be mapped to multiple strain IDs, we used an NCBI genome assembly file
786 (ftp://ftp.ncbi.nlm.nih.gov/genomes/ASSEMBLY_REPORTS/
787 [assembly_summary_genbank.txt](ftp://ftp.ncbi.nlm.nih.gov/genomes/ASSEMBLY_REPORTS/assembly_summary_genbank.txt)) to perform more specific mappings; when more than
788 one mapping was possible, we selected the strain with the oldest genome annotation
789 (we reasoned that strains that were selected first for sequencing were also more likely
790 to have more thorough experimental characterizations). Once SVs were mapped, we
791 restricted our analysis to SVs that grew on a given carbohydrate in over half of the
792 participants and where all BLAST matches to the VMH database had concordant
793 prebiotic utilization annotations. Only consumption of xylan and inulin were examined
794 since GOS and dextrin were not referenced in the VMH database. Permutation analysis

795 were carried out by randomly shuffling rows and columns of the droplet primary
796 degrader table and repeating the analyses.

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799 **Acknowledgements**

800 The authors would like to thank Christopher Mancuso and Ahmad S. Khalil, Ph.D., for
801 their helpful comments on the manuscript. L.A.D. acknowledges support from the
802 Global Probiotics Council, a Searle Scholars Award, an Alfred P. Sloan Research
803 Fellowship, the Beckman Young Investigator program, the Translational Research
804 Institute through Cooperative Agreement NNX16AO69A, the Damon Runyon Cancer
805 Research Foundation, the UNC CGIBD (NIDDK P30DK034987), and NIH
806 1R01DK116187-01. This work used a high-performance computing facility partially
807 supported by grant 2016-IDG-1013 ("HARDAC+: Reproducible HPC for Next-
808 generation Genomics") from the North Carolina Biotechnology Center. *E. coli* strain
809 was provided by N. Lord and J. Paulsson. M.M.V. holds a Postdoctoral Enrichment
810 Program Award from the Burroughs Wellcome Fund. This material is based upon work
811 supported by the National Science Foundation Graduate Research Fellowship under
812 Grant No. DGE-1644868 to R.J.B.

813

814 **Author contributions**

815 M.M.V., R.J.B., and L.A.D. developed the assay platform and designed the study.
816 M.M.V., R.J.B., H.K.D, S.H., S.J. and A.W. performed the experiments. J.D.S.
817 contributed software for bioinformatic analysis. M.M.V., R.J.B., and L.A.D. analyzed the

818 data. S.H. and L.Y. helped with design and data interpretation. M.M.V., R.J.B., and
819 L.A.D. wrote the manuscript with feedback from all authors.

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822 **Competing interests**

823 M.M.V., R.J.B., L.A.D., and Duke University have patents filed related to the droplet
824 platform described herein (PCT/US20 17/045 608, 62/628 170). L.A.D. was a member
825 of the Kaleido Biosciences Strategic Advisory Board and retains equity in the company.