Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

1 **Spatial variation of the native colon microbiota in healthy adults**

2

3

4

5 Running title: Spatial variation of native colon microbiota

6

7 Kaitlin J. Flynn[1], Mack T. Ruffin IV[2], D. Kim Turgeon[3]†, and Patrick D. Schloss[1]†

8 † Corresponding authors: kturgeon@umich.edu and pschloss@umich.edu

9

10 1. Department of Microbiology and Immunology, University of Michigan Medical School, Ann
11 Arbor, Michigan 48109

12 2. Department of Family and Community Medicine, College of Medicine, Pennsylvania State
13 University, Hershey, Pennsylvania 17033

14 3. Department of Internal Medicine, Division of Gastroenterology, University of Michigan Medical
15 School, Ann Arbor, Michigan

16 Grant support: Rose and Lawrence C. Page Foundation

17 Disclosures: nothing to disclose

18 Word count: 3,953/5,000

19 Figures: 6

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

20 **Abstract**

21 The microbiome has been implicated in the development of colorectal cancer and inflammatory
22 bowel diseases. The specific traits of these diseases vary along the axis of the digestive tract.
23 Further, variation in the structure of the gut microbiota has been associated with both diseases.
24 We profiled the microbiota of the healthy proximal and distal mucosa and lumen to better
25 understand how bacterial populations vary along the colon. We used a two-colonoscope
26 approach to sample proximal and distal mucosal and luminal contents from the colons of 20
27 healthy subjects that had not undergone any bowel preparation procedure. The biopsies and
28 home-collected stool were subjected to 16S rRNA gene sequencing and Random Forest
29 classification models were built using taxa abundance and location to identify microbiota specific
30 to each site. The right mucosa and lumen had the most similar community structures of the five
31 sites we considered from each subject. The distal mucosa had higher relative abundance of
32 *Finegoldia, Murdochiella, Peptoniphilus, Porphyromonas* and *Anaerococcus*. The proximal
33 mucosa had more of the genera *Enterobacteriaceae, Bacteroides* and *Pseudomonas*. The
34 classification model performed well when classifying mucosal samples into proximal or distal
35 sides (AUC=0.808). Separating proximal and distal luminal samples proved more challenging
36 (AUC=0.599) and specific microbiota that differentiated the two were hard to identify. By
37 sampling the unprepped colon, we identified distinct bacterial populations native to the proximal
38 and distal sides. Further investigation of these bacteria may elucidate if and how these groups
39 contribute to different disease processes on their respective sides of the colon.

40

41 Keywords: microbiome; colon cancer; proximal and distal colon

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

42 **Introduction**

43 The human colon is an ecosystem comprised of numerous microenvironments that select for
44 different microbiota. Concentrations of oxygen, water, and anti-microbial peptides change along
45 the gut axis and influence which microbiota reside in each location. Microenvironments differ not
46 only longitudinally along the colon, but also radially from the epithelium to mucosa to intestinal
47 lumen, offering several sites for different microbial communities to flourish. The identity of these
48 specific microbiota and communities are important for understanding the etiology of complex
49 diseases such as Colorectal Cancer (CRC) and Inflammatory Bowel Disease (IBD). CRC and
50 IBD can be preceded or accelerated by perturbations of the structure of the gut microbiota (1–
51 3). The manifestations of these diseases are known to vary based upon the location in which
52 they occur. For instance, CRC that arises in the distal (left) colon are of hindgut origin and tend
53 to have large chromosomal alterations indicative of chromosomal instability (1). In contrast,
54 CRC arising in the proximal (right) colon are of midgut origin and tend to be sessile and
55 microsatellite instable (MSI with BRAF and KRAS mutations) (1). In addition to the
56 environmental gradients within the colon, the distal and proximal sides of the colon differ in the
57 amount of inflammation present and the genomic instability of precancerous cells, respectively
58 (1,4,5). In IBD patients, disease occurring in the distal colon extending proximally is usually
59 indicative of ulcerative colitis (UC), whereas Crohn's disease (CD) can occur anywhere along
60 the GI tract, most commonly in the ileum and the cecum (2). UC presents as continuous disease
61 with only mucosal involvement, where as CD has skip lesions and full thickness involvement
62 that may cause abscesses, strictures and fistulas (2). Thus, given the varied physiology of the
63 proximal-distal axis of the colon and known differences in disease patterns at these sites,
64 symbiotic microbiota and their metabolites likely vary as well, and may influence the
65 heterogeneous disease prognoses of IBD and CRC. Because CRC can be a long-term

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

66 complication of IBD, the distribution of microbiota is important to understanding the
67 pathophysiology of both diseases.

68 Several recent findings have shown that development and progression of IBD or CRC can be
69 attributed to specific molecular events as a result of interactions between the gut microbiota and
70 human host (1,3,6). For instance, comparison of the bacteria present on CRC tumors with those
71 found on nearby healthy tissue has identified specific species that are tumor-associated (7).
72 Specific bacteria have also been identified in fecal samples of patients with varying stages of
73 colon tumorigenesis (8,9). These species include the oral pathogens *Fusobacterium nucleatum*
74 and *Porphyromonas asacharolytica*. *F. nucleatum* has also been found to be elevated in the
75 stool and biopsies of patients with IBD as compared to healthy controls (10,11). Furthermore,
76 studies of *F. nucleatum* isolated from mucosal biopsies showed that more invasive *F. nucleatum*
77 positively correlates with IBD disease level (10). Like many intestinal pathogens, the bacteria
78 appear to have a high-impact despite being lowly-abundant in the community (2). The
79 physiology of these rare taxa may contribute to the colonic disease state. These studies often
80 examined only shed human stool or the small intestine, preventing fine-resolution analysis of
81 paired samples from the proximal and distal sides of the colon. Similarly, comparisons of on- or
82 off-tumor/lesion bacteria rarely have matched tissue from the other side of the colon from the
83 same, disease-baring patient, limiting what conclusions can be drawn about the colonic
84 microbiome overall, let alone at that specific site (12). Due to these limitations, the contribution
85 of the gut microbiota to CRC and IBD disease location in the colon is largely undefined.
86 Characterizing these communities in healthy individuals could provide needed insight into
87 disease etiology, including how the disruption of the healthy community could promote the
88 initiation or proliferation of the distinct proximal and distal CRC tumors or IBD flares.

89 The few existing profiles of the microbial spatial variation of the colon have been limited by
90 sample collection methods. The majority of human gut microbiome studies have been

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

91 performed on whole shed feces or on samples collected during colonoscopy or surgery (5).
92 While invasive methods allow investigators to acquire samples from inside the human colon,
93 typically these procedures are preceded by the use of bowel preparation methods such as the
94 consumption of laxatives to cleanse the bowel. Bowel preparation is essential for detecting
95 cancerous or precancerous lesions in the colon, but complicates microbiome profiling as the
96 chemicals strip the bowel of contents and disrupt the mucosal layer (13,14). As such, what little
97 information we do have about the spatial distribution of the microbiota in the proximal and distal
98 colon is confounded by the bowel preparation procedure.

99 Here we address the limitations of previous studies and identify the microbes specific to the
100 lumen and mucosa of the proximal and distal healthy human colon. We used an unprepared
101 colonoscopy technique to sample the natural community of each location of the gut without prior
102 disruption of the native bacteria in 20 healthy volunteers. To address the inherent inter-
103 individual variation in microbiota, we used a machine-learning classification algorithm trained on
104 curated 16S rRNA sequencing reads to identify the microbiota that were specific to each
105 location. We found that our classification models were able to separate mucosal and luminal
106 samples as well as differentiate between sides of the colon based on populations of particular
107 microbiota. By identifying the distinguishing microbiota we are poised to ask if and how the
108 presence or disruption of the microbiota at each site contribute to the development of the tumor
109 subtypes of CRC in the proximal and distal human colon.

110

111 **Methods**

112 **Human subjects**

113 The procedures in this study and consent were approved by the Institutional Review Board at
114 the University of Michigan Health System with protocol number HUM00082721. Subjects were

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

115 recruited using the online recruitment platform and were pre-screened prior to enrollment in the
116 study. Exclusion criteria included: use of asprin or NSAIDs within 7 days, use of antibiotics
117 within 3 months, current use of anticoagulants, known allergies to Fentanyl, Versed and
118 Benadryl, prior history of colon disease, diabetes, abdominal surgery, respiratory, liver, kidney
119 or brain impairments, undergoing current chemotherapy or radiation treatment and subjects that
120 were pregnant or trying to conceive. 20 subjects that met the criteria were selected and
121 provided signed informed consent prior to the procedure. There were 13 female and 7 male
122 subjects ranging in age from 25 to 64. 18 of the 20 subjects had not used antibiotics within a
123 year prior to the collection date and 2 had not used antibiotics within 6 months. None of the
124 subjects had medical conditions requiring frequent or extended antibiotic use.

125 **Sample collection**

126 At a baseline visit, subjects gave consent and were given a home collection stool kit (Zymo).
127 One to seven days prior to the scheduled colonoscopy, subjects collected whole stool at home
128 and shipped the samples to a research coordinator on ice. Notably, subjects did not undergo
129 any bowel preparation method prior to sampling. On the procedure day, subjects reported to the
130 Michigan Clinical Research Unit at the University of Michigan Health System. Subjects were
131 consciously sedated using Fentanyl, Versed and/or Benadryl as appropriate. A flexible
132 sigmoidoscope was first inserted about 25cm into the colon and jumbo biopsy forceps used to
133 collect the luminal contents. Two luminal samples were collected and the contents immediately
134 deposited into RNAlater (Fisher) and flash-frozen in liquid nitrogen. The forceps were withdrawn
135 and new biopsy forceps were used to collect mucosal biopsies on sections of the colon that
136 were pink and free of stool matter. Three mucosal biopsies were collected and flash-frozen in
137 RNAlater. These samples comprised the distal colon samples. The sigmoidoscope was then
138 withdrawn and a pediatric colonoscope was inserted to reach the proximal colon. The proximal
139 samples were taken from the ascending colon proximal to the hepatic flexure at 75-120cm

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

140 depending on the subject. Samples were then collected in the same manner as was done in the
141 distal colon and the colonoscope withdrawn. All samples were stored at -80 °C.

142 **Sample processing, sequencing and analysis**

143 DNA extraction was performed using the PowerMicrobiome DNA/RNA Isolation Kit (MO BIO
144 Laboratories). For tissue biopsies, Bond-Breaker TCEP solution (Fisher) and 2.8mm ceramic
145 beads (MO BIO Laboratories) were added to the bead beating step to enhance DNA recovery
146 from mucosal samples. The resulting DNA was normalized to equal concentrations across all
147 samples and used as template for amplification of the V4 region of the 16S rRNA gene and
148 fragments were sequenced on an Illumina MiSeq as previously described (15). Sequences were
149 curated using the mothur software as described previously (16). The sequences were assigned
150 a taxonomic classification using a naive Bayesian classifier trained using a 16S rRNA gene
151 training set from the Ribosomal Database Project (RDP) (17) and clustered into operational
152 taxonomic units (OTUs) based on a 97% similarity cutoff. Sequencing and analysis of a mock
153 community revealed the error rate to be 0.018%. Samples were rarefied to 4231 sequences per
154 sample in order to reduce the effects of uneven sampling bias.

155 Diversity analysis was performed using the Simpson diversity calculator and θ_{YC} calculator
156 metrics in mothur version 1.39.5 (16). θ_{YC} distances were calculated to determine the
157 dissimilarity between two samples. Random Forest classification models were built using the
158 AUCRF R package using a leave-one-subject out approach (18). The Random Forest models
159 were built using the full, non-rarefied, dataset as input. For each model the data were split into a
160 19-subject training set and a 1-subject test set. The model was built and cross-validated using
161 10-fold k cross-validation (AUCRFcv) on the training set to estimate the prediction error of the
162 model. The resultant model was then used to predict the outcome the left-out subject. This
163 process was repeated iteratively for all subjects and results plotted as Reciever Operator

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

164 Characteristic curves using the pROC R package (19). Resultant models were used to identify
165 the OTUs that were most important for classifying each location. Species-level information for
166 sequences of interest was obtained by aligning the sequences to the GenBank nucleotide
167 databse using blastn. The species name was only used if the identity score was ≥ 99% over the
168 full-length of the contig and matched a single reference.

169 **Statistical analysis**

170 Differences in community membership at the phyla level were tested using the analysis of
171 molecular variance (AMOVA) metric in mothur. Differences in θ_{YC} distances by location were
172 tested using the Wilcoxon rank-sum test adjusted for multiple comparisons using the Benjamini-
173 Hochberg procedure.

174 **Data availability**

175 16S rRNA gene sequence reads and experiment metadata are available on the NCBI Sequence
176 Read Archive (SRA) with accession number SRP124947 and PRJNA418115. A reproducible
177 data analysis pipeline can be found at
178 https://github.com/SchlossLab/Flynn_LRColon_CancPrevRes_2017.

179

180 **Results**

181 **Microbial membership and diversity of the proximal and distal colon**

182 Luminal and mucosal samples were collected from the proximal and distal colon of 20 healthy
183 individuals who had not undergone bowel preparation (Fig. 1). Subjects also collected stool at
184 home one week prior to the procedure. To characterize the bacterial communities present at
185 these sites, 16S rRNA gene sequencing was performed on DNA extracted from each sample.
186 As expected, each site was primarily dominated by *Firmicutes* and *Bacteriodetes* (Fig. 2A) (20).

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

187 Samples had varying levels of diversity at each site, irrespective of the individual (Fig. 2B). For
188 example, the proximal mucosa was more diverse than the distal for some individuals while the
189 opposite was true for others. Therefore we could not identify a clear pattern of changes in
190 microbial diversity along the gut axis.

191 To compare similarity between the proximal and distal sides and within the lumen and mucosa,
192 we compared the community structure of these sites based on the relative abundances of
193 individual Operational Taxonomic Units (OTUs). Across all subjects we observed wide variation
194 when comparing sample locations (Fig. 3A). Those ranges did not follow a clear pattern on an
195 individual basis. However, when comparing median dissimilarity between the communities
196 found in the proximal lumen and mucosa, the proximal and distal lumen, the proximal and distal
197 mucosa, and the distal lumen and mucosa, we found that the proximal lumen and mucosa were
198 most similar to each other than to the other samples ($P < 0.005$, Wilcoxon, BH adjustment).

199 **Fecal samples resemble luminal samples from the distal colon**

200 Next, we compared the luminal and mucosal samples to the fecal sample of each subject.
201 Amidst the large inter-subject variation, we did identify significantly less dissimilarity between
202 the distal luminal sample and the feces (Fig. 3B, $P < 0.05$, Wilcoxon, BH adjustment).
203 Furthermore, there was an even larger difference in the communities found in the distal mucosa
204 compared to the fecal communities, indicating that the mucosa is as different from the stool as
205 compared to lumen ($P < 0.0005$, Wilcoxon, BH adjustment). These results suggest that the
206 contents of the distal lumen were most representative of the subjects' feces, and the mucosal
207 microbiota are distinct from the fecal and luminal communities.

208 **Interpersonal community variation is greater than the variation between sites**

209 To determine what factors may have driven the differences seen among the samples, we
210 compared the community dissimilarity between samples from all subjects (interpersonal) versus

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

211 samples from within one subject (intrapersonal). We found that samples from one individual
212 were far more similar to each other than to matched samples from the other subjects (Fig. 3C);
213 this is consistent with previous human microbiome studies that have sampled multiple sites of
214 the human colon (21–23). Thus interpersonal variation drove the differences between samples
215 more than whether the sample came from the proximal or distal side of the colon or from the
216 lumen or mucosa.

217 **Random Forest classification models identify important OTUs on each side**

218 To identify OTUs that were distinct at each site, we constructed several Random Forest models
219 trained using OTU relative abundances. We built the first model to classify the luminal versus
220 mucosal samples for the proximal and distal sides, independently (Fig. 4A). The models
221 performed well when classifying these samples (proximal AUC = 0.716, distal AUC = 0.862).
222 The OTUs that were most predictive of each site were identified by their greatest mean
223 decrease in accuracy when removed from the model. For distinguishing the proximal lumen and
224 mucosa, OTUs affiliated with the *Bacteriodes*, *Actinomyces*, *Psuedomonas* and
225 *Enterobacteraceae* were included in the best model (Fig. 5A). The model to differentiate
226 between the distal lumen and mucosa included OTUs affiliated with the *Turicibacter*, *Finegoldia*,
227 *Peptoniphilus* and *Anaerococcus* (Fig. 5B). These results indicated that there were fine
228 differences between the different sites of the colon, and that these could be traced to specific
229 OTUs on each side.

230 Next, we built a Random Forest model to differentiate the proximal and distal luminal samples.
231 The model performed best when distinguishing the proximal versus distal mucosa (Fig. 4B, AUC
232 = 0.808) whereas the model to differentiate between the proximal versus distal lumen performed
233 poorly (AUC = 0.599). The OTUs included in the model differentiating the distal and proximal
234 mucosa included members of the *Porphyromonas*, *Murdochiella*, *Finegoldia*, *Anaerococcus* and
235 *Peptoniphilus* (Fig. 6A). The model that attempted to separate the the proximal and distal lumen

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

236 included OTUs affiliated with the *Bacteroides*, *Clostridium IV* and *Oscillibacter* (Fig. 6B).
237 Interestingly, *Anaerococcus* and *Finegoldia* were distinct between the mucosa and lumen and
238 also helped to differentiate between the proximal and distal sides.

239 **Bacterial OTUs associated with CRC and IBD are found in healthy individuals**

240 Given that specific bacterial species have been associated with colorectal cancer and IBD, we
241 probed our sample set for these OTUs. Among our 100 samples, the most frequent sequence
242 associated with the *Fusobacterium* genus was OTU179, which aligned via blastn to
243 *Fusobacterium nucleatum subsp animalis* (100% over full length). This is the only species of
244 *Fusobacterium* known to have oncogenic properties and be found on the surfaces of colorectal
245 cancer tumors (24). There were 14 samples from 8 subjects with the *F. nucleatum subsp.*
246 *animalis* sequences. Of the samples with the highest relative abundance of *F. nucleatum subsp.*
247 *animalis*, four of the samples were from the proximal mucosa and three from the distal mucosa
248 (Supplementary Fig. S1A). The second most frequent *Fusobacterium* sequence was OTU472,
249 which aligned with 99% identity to *F. varium*. In addition to *F. nucleatum*, *F. varium* has been
250 associated with IBD (25). Four subjects harbored *F. varium* and the samples were split evenly
251 between the proximal and distal mucosa (Supplementary Fig. S1B). OTU152 was similar to the
252 members of the *Porphyromonas* genus and the most frequent sequence in that OTU aligned to
253 *Porphyromonas asacharolytica* (99% over full length), another bacterium commonly detected
254 and isolated from colorectal tumors. OTU152 was only detected on the distal mucosa, and in
255 fact was one of the OTUs the classification model identified as separating distal and proximal
256 sides (Supplementary Fig. S1C). Among the 11 distal mucosa samples that were positive for *P.*
257 *asacharolytica*, the relative abundances for this OTU ranged from 0.01% to 16%. Thus, disease-
258 associated OTUs could be found in our sample set of 20 healthy individuals.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

259 **Discussion**

260 We identified bacterial taxa that were specific to the lumen and mucosa of the proximal and
261 distal sides of the human colon using samples collected during an unprepared colonoscopy of
262 healthy subjects. We found that all locations contained a range of phylum relative abundances
263 and a range of diversity, but that there was a wide variability between subjects. Pairwise
264 comparisons of each of the sites revealed that the proximal mucosa and lumen were most
265 similar to each other. Further, comparison of colonoscopy-collected samples with fecal samples
266 demonstrated that the distal lumen was most similar to feces. Random Forest models built using
267 OTU relative abundances from each sample identified microbiota that were particular to each
268 location of the colon. Finally, we were able to detect some bacterial OTUs associated with
269 colonic disease in our healthy cohort. Using unprepped colonoscopies and machine learning,
270 we have identified bacterial taxa specific to the healthy proximal and distal human colon.

271 When examining the relative abundance of the dominant phyla at each site (i.e. *Bacteriodes* and
272 *Firmicutes*), there was a wide amount of variation. This likely reflects not only the variability
273 between human subjects, caused by differences in age, sex, and diet, but may also reflect
274 spatial patchiness in the gut microbiome within a subject. Patchiness refers to inconsistent
275 distribution of microbial populations due to fluctuations in local resources (26). One study noted
276 that the bacteria recoverable from the same mucosal sample location can be vastly different
277 when the samples are taken just 1 cm away from each other (27). Similar patchiness was also
278 observed in luminal contents and fecal samples themselves; there was separation of different
279 interacting microbes along the length of a stool sample, for instance (28). A third study that
280 sampled six mucosal sites along the colon observed such patchiness in two of the three study
281 subjects (21). While our subjects were not sampled frequently enough to draw specific
282 conclusions about patchiness along the unprepped colon, we did observe some specific
283 differences in mucosal versus luminal samples at the phylum level. The mucosal samples

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

284 harbored more *Proteobacteria*, consistent with previous studies comparing mucosal swabs to
285 luminal content in humans (4). However, we must still consider that the results from phyla
286 analysis may have been impacted by inter-subject patchiness.

287 To get around the noisiness from a diverse set of samples, we built Random Forest
288 classification models to identify the microbiota that were specific to each side and in the lumen
289 and mucosa. For each comparison we identified the top five OTUs that were strongly predictive
290 of one site or another. Generally, OTUs identified in each location were consistent with known
291 physiological gradients along the gut axis (5). For instance, the proximal mucosa contains the
292 highest oxygen concentrations of the colon and harbored mucosa-associated facultative
293 anaerobes such as *Actinomyces* and *Enterobacteraceae* and aerobic *Psuedomonas*. The distal
294 mucosa was far more likely to host strictly anaerobic species such as *Porphyromonas*,
295 *Anaerococcus*, *Finegoldia* and *Peptoniphilus*. Thus the gut microenvironment of each location
296 likely enriches for these specific microbiota.

297 In addition to identifying features that are specific to each side of the gut, the ability of the
298 Random Forest to classify samples can serve as a proxy for similarity. That is, a higher AUC
299 value indicates the samples are more efficiently classfied (and thus more different) than a model
300 with a lower AUC value. For instance, the model separating the proximal and distal mucosa had
301 an AUC of 0.850 whereas the model for classifying the proximal and distal lumen had a much
302 lower AUC of 0.580. Further, the latter model required 44 OTUs to best separate the samples
303 whereas the models separating the mucosa only needed 10 OTUs. The much lower AUC and
304 need for a high number of features compared to other models suggest these locations are the
305 most similar of the comparisons tested. We speculate that the model was less effective at
306 classifying the proximal and distal luminal contents because the mucosal microenvironments
307 have more variable selective pressure along the colon than the luminal microenvironments.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

308 We detected *F. nucleatum* and *P. asacharolytica* in 8 and 5 of our subjects, respectively. These
309 bacteria have been shown to be predictive of colorectal cancer in humans (9) and have
310 oncogenic properties in cell culture and in mice (29). Though the bacteria are known to co-
311 localize on CRC tumors, in our study *F. nucleatum* was found on both sides of the colon while
312 *P. asacharolytica* was only detected in the distal mucosa. Not much is known about the
313 distribution of *P. asacharolytica* along the healthy colon, but given its anaerobic lifestyle and
314 asacharolytic metabolism, it is perhaps not surprising that our study detected the bacteria
315 primarily in the less-oygen-rich and protein-rich distal mucosa (4). In studies examining bacteria
316 on colorectal cancer tumors, *F. nucleatum* was more commonly detected on proximal-sided
317 tumors, and distribution of *F. nucleatum* decreased along the colon to rectum (30). Of the 8
318 (40%) individuals positive for *F. nucleatum* in our study, the bacterium was spread across the
319 proximal mucosa, distal lumen and distal mucosa. The *Fusobacterium* species *nucleatum* and
320 *varium* have been commonly isolated from mucosal biopsies of patients with IBD and UC
321 (25,31). In our study, *F. varium* was only detected in three subjects and two of those samples
322 were isolated form the proximal mucosa (Supplementary Fig. S1B). *F. varium* is most commonly
323 isolated from UC patient biopsies from the ileum or cecum (adjacent to the proximal colon) (32),
324 suggesting this species may exhibit preference for the higher oxygen content of these
325 gastrointestinal sites.

326 Spatial organization of *Fusobacteria* and other bacterial species into polymicrobial biofilms that
327 can invade the gut mucosa have been linked to CRC (33). The biofilms promote tumorigenesis
328 by allowing bacteria to grow near the epithelium, inducing inflammation, genotoxicity and
329 metabolic changes that favor tumor cell growth (33). In one study of CRC biofilms and tumors,
330 all of the proximal tumors examined contained a polymicrobial biofilm on the tumor mucosa (7).
331 Further examination of these tumors identified *Fusobacteria* species as members of the biofilm.
332 These results indicate that it is not only the presence of the bacterium but the tumor community

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

333 as a whole that contributes to tumorigenesis (7). A 'driver-passenger' model has been proposed
334 as a mechanism for biofilm assembly in the gut (34,35). In this model, 'driver' species such as
335 *Fusobacterium spp* and *Porphyromonas spp* exert tumorigenic effects locally and create a niche
336 for adherence of 'passenger' species that comprise the rest of the biofilm (34,35). Thus the the
337 distribution of these disease-associated microbes in healthy patients is of interest as their
338 presence can be predictive of disease prior to the onset of symptoms (9). A better
339 understanding of the early microbial changes in the gut microboiome is essential for elucidating
340 a mechanism for development of CRC or IBD subtypes in the proximal or distal colon.

341 Specific comparisons of our findings to previously published studies of spatial variation are
342 confounded by the use of bowel preparation methods. A rare report of a matched-colonoscopy
343 study sampled 18 patient's colonic mucosa and luminal contents prior to and after bowel
344 cleansing (36). This study found that mucosal and luminal samples were distinguishable prior to
345 bowel cleansing, but that bowel preparation resulted in an increase in shared OTUs between
346 each site (36). After seven days, bowel cleansing not only made the samples more difficult to
347 distinguish, but it also decreased the diversity observed across sites. Bowel preparation clearly
348 biases the representation of microbiota recovered from sampling the lumen or mucosa.

349 By revealing specific differences in microbial populations at each location in the gut via sampling
350 an unprepared bowel, we can begin to form hypotheses about how specific host-microbe
351 interactions can affect disease progression of proximal and distal CRC and IBD subtypes.
352 Future investigation of these samples using metagenomics and metatranscriptomics would
353 illuminate the microbial activities in these gut microenvironments. Further, combining this
354 approach with a more comprehensive sampling strategy along the unprepped colon could
355 enhance microbiome-based screening and treatment modalities for colon disease.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

356 **Acknowledgments**

357 We thank all the individuals who volunteered for the study. This work was supported by the
358 Rose and Lawrence C. Page Foundation (DKT). We would also like to thank Brian Kleiner,
359 Chelsea Crofoot, and Kirk Herman for their roles in study coordination, subject recruitment,
360 sample collection and sample processing. Thanks also to GI Fellows Drs Amy Hosmer,
361 Alexander Larson and Anna Tavakkoli who assisted Dr. Turgeon with the colonoscopies.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

362 **References**

363 1. Yamauchi M, Lochhead P, Morikawa T, Huttenhower C, Chan AT, Giovannucci E, et al.
364 Colorectal cancer: A tale of two sides or a continuum?: Figure 1. Gut [Internet]. BMJ;
365 2012;61:794–7. Available from: https://doi.org/10.1136/gutjnl-2012-302014

366 2. Forbes JD, Domselaar GV, Bernstein CN. The gut microbiota in immune-mediated
367 inflammatory diseases. Frontiers in Microbiology [Internet]. Frontiers Media SA; 2016;7.
368 Available from: https://doi.org/10.3389/fmicb.2016.01081

369 3. Halfvarson J, Brislawn CJ, Lamendella R, Vazquez-Baeza Y, Walters WA, Bramer LM, et al.
370 Dynamics of the human gut microbiome in inflammatory bowel disease. Nature Microbiology
371 [Internet]. Springer Nature; 2017;2:17004. Available from:
372 https://doi.org/10.1038/nmicrobiol.2017.4

373 4. Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, et al. Correlation
374 between intraluminal oxygen gradient and radial partitioning of intestinal microbiota.
375 Gastroenterology [Internet]. Elsevier BV; 2014;147:1055–1063.e8. Available from:
376 https://doi.org/10.1053/j.gastro.2014.07.020

377 5. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota.
378 Nature Reviews Microbiology [Internet]. Springer Nature; 2015;14:20–32. Available from:
379 https://doi.org/10.1038/nrmicro3552

380 6. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium
381 nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune
382 microenvironment. Cell Host & Microbe [Internet]. Elsevier BV; 2013;14:207–15. Available from:
383 https://doi.org/10.1016%2Fj.chom.2013.07.007

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

384 7. Dejea CM, Wick EC, Hechenbleikner EM, White JR, Welch JLM, Rossetti BJ, et al.
385 Microbiota organization is a distinct feature of proximal colorectal cancers. Proceedings of the
386 National Academy of Sciences [Internet]. Proceedings of the National Academy of Sciences;
387 2014;111:18321–6. Available from: https://doi.org/10.1073/pnas.1406199111

388 8. McCoy AN, Araújo-Pérez F, Azcárate-Peril A, Yeh JJ, Sandler RS, Keku TO. Fusobacterium
389 is associated with colorectal adenomas. Goel A, editor. PLoS ONE [Internet]. Public Library of
390 Science (PLoS); 2013;8:e53653. Available from:
391 https://doi.org/10.1371%2Fjournal.pone.0053653

392 9. Baxter NT, Ruffin MT, Rogers MAM, Schloss PD. Microbiota-based model improves the
393 sensitivity of fecal immunochemical test for detecting colonic lesions. Genome Medicine
394 [Internet]. Springer Nature; 2016;8. Available from: https://doi.org/10.1186/s13073-016-0290-3

395 10. Strauss J, Kaplan GG, Beck PL, Rioux K, Panaccione R, DeVinney R, et al. Invasive
396 potential of gut mucosa-derived fusobacterium nucleatum positively correlates with IBD status of
397 the host. Inflammatory Bowel Diseases [Internet]. Ovid Technologies (Wolters Kluwer Health);
398 2011;17:1971–8. Available from: https://doi.org/10.1002/ibd.21606

399 11. Brennan CA, Garrett WS. Gut microbiota, inflammation, and colorectal cancer. Annual
400 Review of Microbiology [Internet]. Annual Reviews; 2016;70:395–411. Available from:
401 https://doi.org/10.1146%2Fannurev-micro-102215-095513

402 12. Flemer B, Lynch DB, Brown JMR, Jeffery IB, Ryan FJ, Claesson MJ, et al. Tumour-
403 associated and non-tumour-associated microbiota in colorectal cancer. Gut [Internet]. BMJ
404 Publishing Group; 2017;66:633–43. Available from: http://gut.bmj.com/content/66/4/633

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

405 13. Jalanka J, Salonen A, Salojärvi J, Ritari J, Immonen O, Marciani L, et al. Effects of bowel
406 cleansing on the intestinal microbiota. Gut [Internet]. BMJ; 2014;64:1562–8. Available from:
407 https://doi.org/10.1136/gutjnl-2014-307240

408 14. Harrell L, Wang Y, Antonopoulos D, Young V, Lichtenstein L, Huang Y, et al. Standard
409 colonic lavage alters the natural state of mucosal-associated microbiota in the human colon.
410 Singh SR, editor. PLoS ONE [Internet]. Public Library of Science (PLoS); 2012;7:e32545.
411 Available from: https://doi.org/10.1371/journal.pone.0032545

412 15. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-
413 index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the
414 MiSeq illumina sequencing platform. Applied and Environmental Microbiology [Internet].
415 American Society for Microbiology; 2013;79:5112–20. Available from:
416 https://doi.org/10.1128/aem.01043-13

417 16. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing
418 mothur: Open-source, platform-independent, community-supported software for describing and
419 comparing microbial communities. Applied and Environmental Microbiology [Internet]. American
420 Society for Microbiology; 2009;75:7537–41. Available from: https://doi.org/10.1128/aem.01541-
421 09

422 17. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive bayesian classifier for rapid assignment of
423 rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology
424 [Internet]. American Society for Microbiology; 2007;73:5261–7. Available from:
425 https://doi.org/10.1128/aem.00062-07

426 18. Calle ML, Urrea V, Boulesteix A-L, Malats N. AUC-RF: A new strategy for genomic profiling
427 with random forest. Human Heredity [Internet]. S. Karger AG; 2011;72:121–32. Available from:
428 https://doi.org/10.1159%2F000330778

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

429 19. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, et al. pROC: An open-
430 source package for r and s to analyze and compare ROC curves. BMC Bioinformatics [Internet].
431 Springer Nature; 2011;12:77. Available from: https://doi.org/10.1186%2F1471-2105-12-77

432 20. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome
433 Medicine [Internet]. Springer Nature; 2016;8. Available from: https://doi.org/10.1186/s13073-
434 016-0307-y

435 21. Eckburg PB. Diversity of the human intestinal microbial flora. Science [Internet]. American
436 Association for the Advancement of Science (AAAS); 2005;308:1635–8. Available from:
437 https://doi.org/10.1126/science.1110591

438 22. Cárcer DA de, Cuív PÓ, Wang T, Kang S, Worthley D, Whitehall V, et al. Numerical ecology
439 validates a biogeographical distribution and gender-based effect on mucosa-associated bacteria
440 along the human colon. The ISME Journal [Internet]. Springer Nature; 2010;5:801–9. Available
441 from: https://doi.org/10.1038/ismej.2010.177

442 23. Zhang Z, Geng J, Tang X, Fan H, Xu J, Wen X, et al. Spatial heterogeneity and co-
443 occurrence patterns of human mucosal-associated intestinal microbiota. The ISME Journal
444 [Internet]. Springer Nature; 2013;8:881–93. Available from:
445 https://doi.org/10.1038/ismej.2013.185

446 24. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, et al.
447 Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome
448 Research [Internet]. Cold Spring Harbor Laboratory Press; 2011;22:299–306. Available from:
449 https://doi.org/10.1101/gr.126516.111

450 25. Lee Y, Eun CS, Lee AR, Park CH, Han DS. FusobacteriumIsolates recovered from colonic
451 biopsies of inflammatory bowel disease patients in korea. Annals of Laboratory Medicine

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

452 [Internet]. Korean Society for Laboratory Medicine (KAMJE); 2016;36:387. Available from:
453 https://doi.org/10.3343/alm.2016.36.4.387

454 26. Rougharden JD. Patchiness in the spatial distribution of a population caused by stochastic
455 fluctuations in resources. Oikos [Internet]. JSTOR; 1977;29:52. Available from:
456 https://doi.org/10.2307/3543292

457 27. Hong P-Y, Croix JA, Greenberg E, Gaskins HR, Mackie RI. Pyrosequencing-based analysis
458 of the mucosal microbiota in healthy individuals reveals ubiquitous bacterial groups and micro-
459 heterogeneity. Ahmed N, editor. PLoS ONE [Internet]. Public Library of Science (PLoS);
460 2011;6:e25042. Available from: https://doi.org/10.1371/journal.pone.0025042

461 28. Stearns JC, Lynch MDJ, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, et
462 al. Bacterial biogeography of the human digestive tract. Scientific Reports [Internet]. Springer
463 Nature; 2011;1. Available from: https://doi.org/10.1038/srep00170

464 29. Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. Cell Host & Microbe
465 [Internet]. Elsevier BV; 2014;15:317–28. Available from:
466 https://doi.org/10.1016/j.chom.2014.02.007

467 30. Mima K, Cao Y, Chan AT, Qian ZR, Nowak JA, Masugi Y, et al. Fusobacterium nucleatum in
468 colorectal carcinoma tissue according to tumor location. Clinical and Translational
469 Gastroenterology [Internet]. Springer Nature; 2016;7:e200. Available from:
470 https://doi.org/10.1038/ctg.2016.53

471 31. Ohkusa T. Induction of experimental ulcerative colitis by fusobacterium varium isolated from
472 colonic mucosa of patients with ulcerative colitis. Gut [Internet]. BMJ; 2003;52:79–83. Available
473 from: https://doi.org/10.1136/gut.52.1.79

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

474 32. Ohkusa T, Sato N, Ogihara T, Morita K, Ogawa M, Okayasu I. Fusobacterium varium
475 localized in the colonic mucosa of patients with ulcerative colitis stimulates species-specific
476 antibody. Journal of Gastroenterology and Hepatology [Internet]. Wiley-Blackwell; 2002;17:849–
477 53. Available from: https://doi.org/10.1046/j.1440-1746.2002.02834.x

478 33. Li S, Konstantinov SR, Smits R, Peppelenbosch MP. Bacterial biofilms in colorectal cancer
479 initiation and progression. Trends in Molecular Medicine [Internet]. Elsevier BV; 2017;23:18–30.
480 Available from: https://doi.org/10.1016/j.molmed.2016.11.004

481 34. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driverpassenger model for
482 colorectal cancer: Beyond the usual suspects. Nature Reviews Microbiology [Internet]. Springer
483 Nature; 2012;10:575–82. Available from: https://doi.org/10.1038/nrmicro2819

484 35. Flynn KJ, Baxter NT, Schloss PD. Metabolic and community synergy of oral bacteria in
485 colorectal cancer. McMahon K, editor. mSphere [Internet]. American Society for Microbiology;
486 2016;1:e00102–16. Available from: https://doi.org/10.1128/msphere.00102-16

487 36. Shobar RM, Velineni S, Keshavarzian A, Swanson G, DeMeo MT, Melson JE, et al. The
488 effects of bowel preparation on microbiota-related metrics differ in health and in inflammatory
489 bowel disease and for the mucosal and luminal microbiota compartments. Clinical and
490 Translational Gastroenterology [Internet]. Springer Nature; 2016;7:e143. Available from:
491 https://doi.org/10.1038/ctg.2015.54

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

492 **Figure legends**

493 **Figure 1**

494 Sampling strategy. A flexible sigmoidoscope was used to sample the distal colonic luminal
495 contents and mucosa. The scope was inserted ~ 25cm into the subject and biopsy forceps were
496 used to sample the luminal contents (D, inset). A separate set of biopsy forceps was used to
497 sample the distal mucosa (D, inset). The sigmoidoscope was removed. A pediatric colonoscope
498 was inserted and used to access the proximal colon (P, inset). Biopsies were taken of the
499 proximal luminal contents and mucosa as described. One week prior to the procedure stool was
500 collected at home and sent into the laboratory. Representative images from one individual are
501 shown.

502 **Figure 2**

503 Phylum-level relative abundance and diversity in the proximal and distal human colon. A)
504 Relative abundance of the top five bacterial phyla in each sampling site. Each box represents
505 the median and interquartile range. B) Simpson diversity of the microbial communities at each
506 location. The horizontal lines represent the median values.

507 **Figure 3**

508 Comparison of microbial community structure between sites of the gut. θ_{YC} distances are shown
509 to indicate the interpersonal dissimilarities between two sites – each point represents one
510 individual. In (A), comparisons of the proximal and distal mucosal and lumen are shown. In (B),
511 comparisons of each site to the exit stool are shown. In (C), comparisons of samples from all
512 subjects to each other (interpersonal) or within one subject (intrapersonal) are shown.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

513 **Figure 4**

514 Random Forest classifies locations in the colon. A) Receiver Operator Characteristic curves are
515 shown for the Random Forest model classifying lumen and mucosal samples for the distal (red)
516 and proximal (blue) sides of the colon. (B) Receiver Operator Characteristic curves are shown
517 for the 10-fold cross validation of the Random Forest model classifying distal mucosa vs
518 proximal mucosa (green) and distal lumen versus proximal lumen (purple).

519 **Figure 5**

520 Taxa specific to the distal and proximal sides of the colon. Top five OTUs that are most
521 important for the classification model for the distal mucosa and lumen (A) and the proximal
522 mucosa and lumen (B). The vertical lines represent the median values for each OTU.

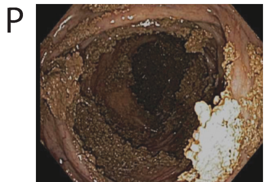
523 **Figure 6**

524 Taxa specific to the distal and proximal mucosa and lumen. The five OTUs that were most
525 important differentiating the distal and proximal mucosa (A) and the distal and proximal lumen
526 (B). The vertical lines represent the median values for each OTU.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.

P

D



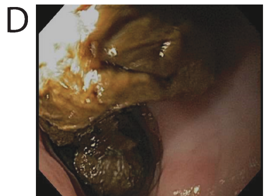


Figure 1

Downloaded from cancerpreventionresearch.aacrjournals.org on May
Cancer Research.

A

% Relative Abundance

75
50
25
0

P Mucosa
P Lumen
D Mucosa
D Lumen
Stool

Phylum
Firmicutes
Bacteroidetes
Proteobacteria
Verrucomicrobia
Actinobacteria
Fusobacteria

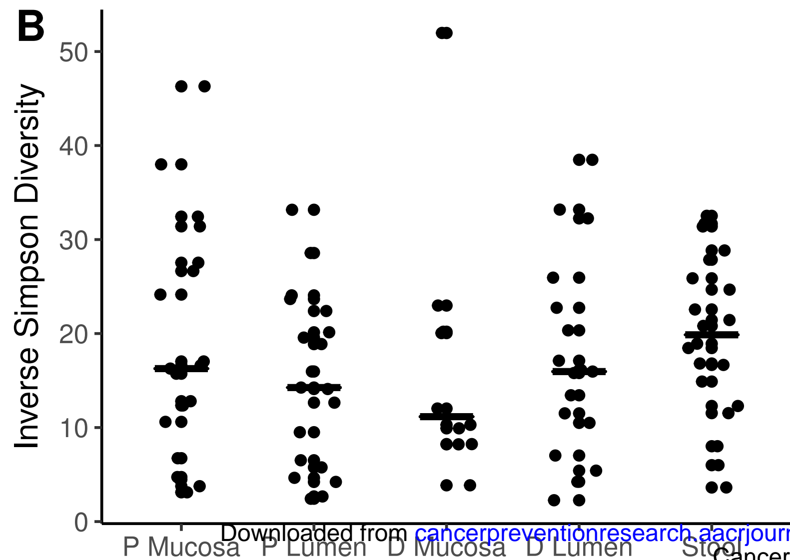
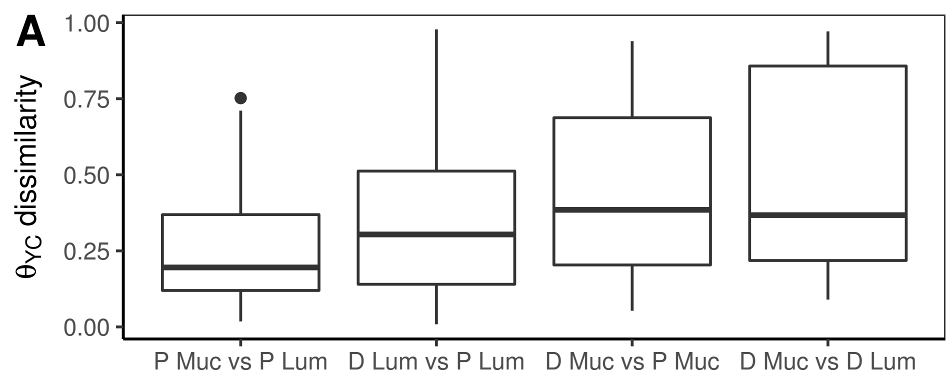
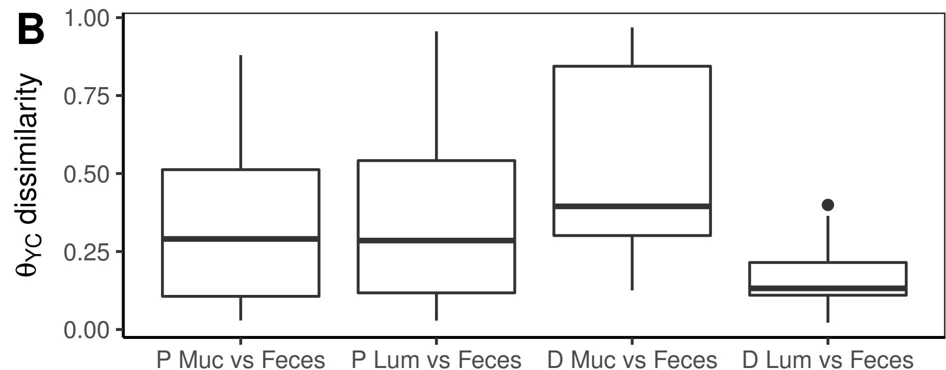


Figure 2

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 A
Cancer Research.





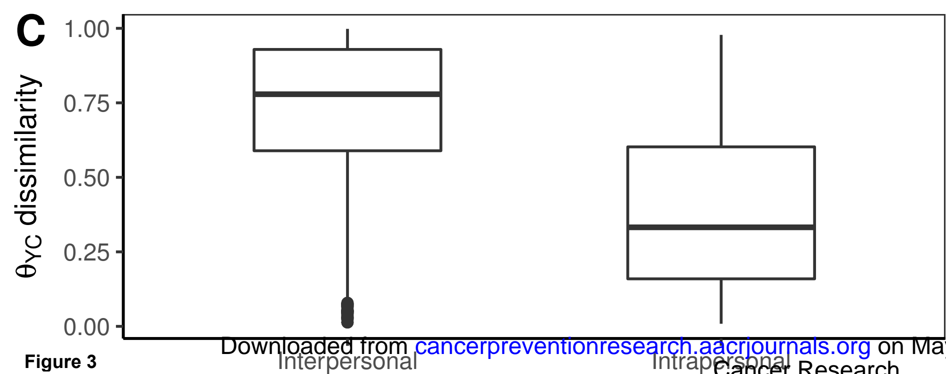


Figure 3

Downloaded from cancerpreventionresearch.aacrjournals.org on May
Cancer Research.

A

D Lum vs D Muc, AUC = 0.863
P Lum vs P Muc, AUC = 0.716

Sensitivity

Specificity

B

D Muc vs P Muc, AUC = 0.808
D Lum vs P Lum, AUC = 0.599

Sensitivity

Specificity

Figure 4

Downloaded from cancerpreventionresearch.aacrjou
Canc

A

P Muc
P Lum

Enterobacteriaceae (OTU 87)
Bacteroides (OTU 20)
Actinomyces (OTU 99)
Enterobacteriaceae (OTU 38)
Pseudomonas (OTU 94)

0 0.1 1 10 100

Relative Abundance (%)

B

D Muc
D Lum

Turicibacter (OTU 138)
Anaerococcus (OTU 143)
Finegoldia (OTU 340)
Anaerococcus (OTU 144)
Peptoniphilus (OTU 129)

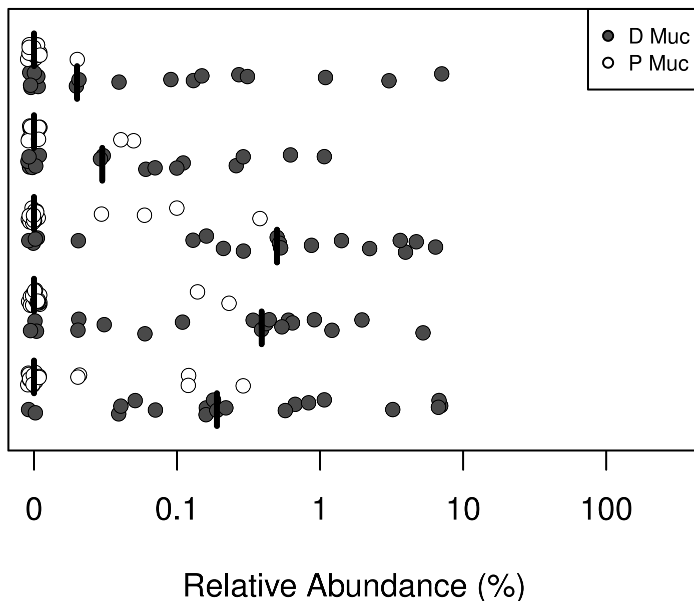
0 0.1 1 10 100

Relative Abundance (%)

Figure 5

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 A
Cancer Research.

A



B

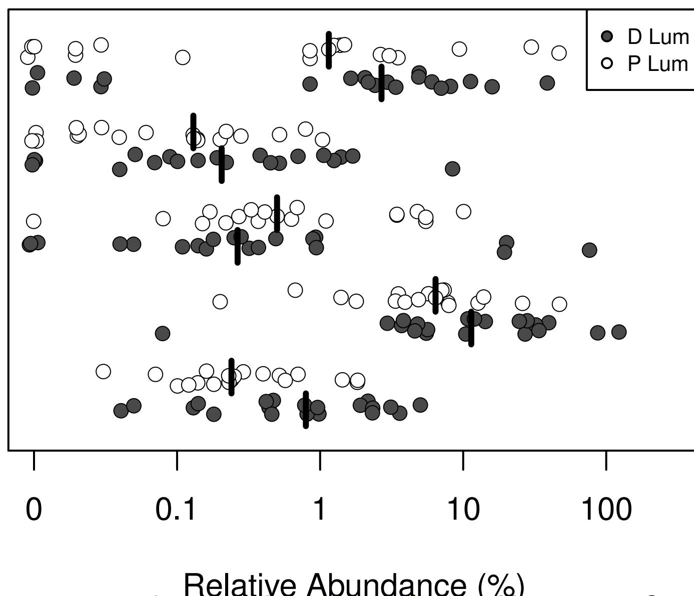


Figure 6

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 A
Cancer Research.

Author Manuscript Published OnlineFirst on April 10, 2018; DOI: 10.1158/1940-6207.CAPR-17-0370
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

AACR American Association for Cancer Research

Cancer Prevention Research

Spatial variation of the native colon microbiota in healthy adults

Kaitlin J Flynn, Mack T Ruffin, D. Kim Turgeon, et al.

Cancer Prev Res Published OnlineFirst April 10, 2018.

Updated version Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-17-0370

Supplementary Material Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2018/04/10/1940-6207.CAPR-17-0370.DC1

Author Manuscript Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
http://cancerpreventionresearch.aacrjournals.org/content/early/2018/04/10/1940-6207.CAPR-17-0370.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 9, 2018. © 2018 American Association for Cancer Research.