NAME OF THIS STUDY

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Abstract

Introduction

Results and Discussion

Scaling up. The advantage of the dual-index approach is that a large number of samples can be sequenced using a number of primers equal to only twice the square root of the number of samples. To fully evaluate this approach, we resequenced the V4 region of 360 samples that were previously described by sequencing the distal end of the V35 region on the 454 GS-FLX Titanium platform (1). In that study, we observed a clear separation between murine fecal samples obtained from 8 C57BL/6 mice at 0 to 9 (early) and 141 to 150 (late) days after weaning, and there was significantly less variation between the late samples than the early samples. In addition to the mouse fecal samples, we allocated 2 pairs of indices to resequence our mock community. We generated 3.9 million pairs of sequence reads from the 16S rRNA gene with an average coverage of 10,755.9 pairs of reads per sample (95% of the samples had more than 2,788.2 pairs of sequences) using a new collection of 8-nt indices (see the supplemental material). Although individual samples were expected to have various amplification efficiencies, analysis of the number of reads per index did not suggest a systematic positive or negative amplification bias that could be attributed to the indices. The combined error rate for the two mock communities was 0.07% before preclustering and 0.01% after (n = 14,094 sequences). When we used UCHIME to remove chimeras and rarefied to 5,000 sequences, there was an average of 30.4 OTUs (i.e., 10.4 spurious OTUs). Similar to our previous results, ordination of the mouse fecal samples again showed the separation between the early and late periods and increased stabilization with age (Fig. 4) (Mantel test coefficient, 0.81; $P < 0.001$). These results clearly indicate that our approach can be scaled to multiplex large numbers of samples.

Conclusions

Materials and Methods

(Fig. 4) Principal coordinate ordination of YC values (2) relating the community structures of the fecal microbiota from 12 mice collected on days 0 through 9 (Early) and days 141 through 150 (Late) after weaning.

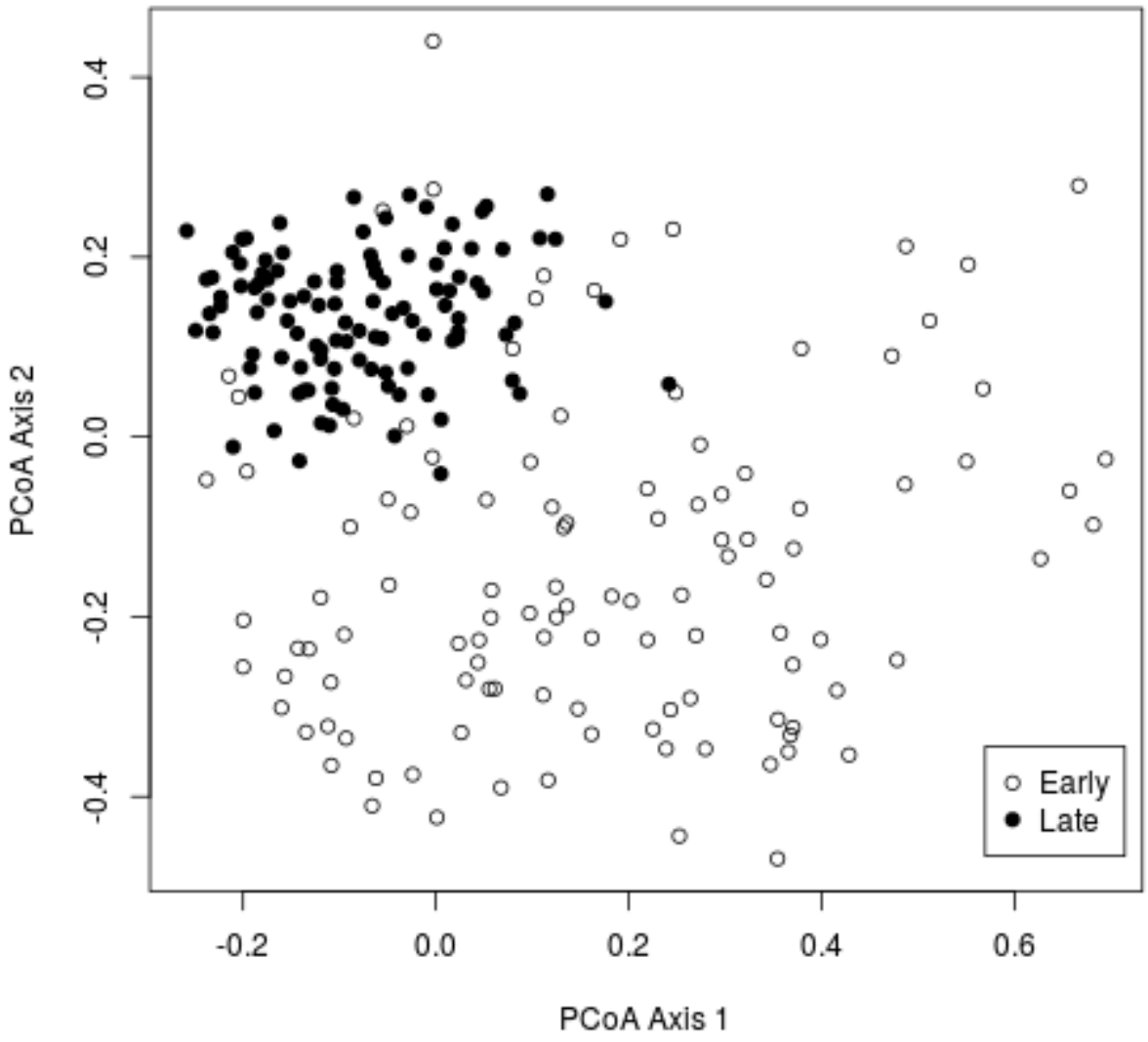

Figure 1:

Day	Seqs. persample (mean)	Seqs. perday (total)	Number ofsamples
0	11403.8	136845	12
1	8672.0	104064	12

Day	Seqs. persample (mean)	Seqs. perday (total)	Number ofsamples
2	11378.2	136539	12
3	14851.5	178218	12
4	13870.8	152579	11
5	10356.9	124283	12
6	11316.7	135800	12
7	11996.2	143954	12
8	9247.5	101723	11
9	10798.9	129587	12
11	13717.9	150897	11
13	15780.6	173587	11
15	8723.9	95963	11
17	8908.5	89085	10
19	7021.9	70219	10
21	11461.9	114619	10
25	9184.2	110210	12
45	8290.8	66326	8
65	12785.9	153431	12
124	19060.7	57182	3
125	11424.1	102817	9
141	8202.4	98429	12
142	10668.0	128016	12
143	15228.6	182743	12
144	10835.7	130028	12
145	10362.3	124348	12
146	7528.8	90345	12
147	9929.2	119151	12
148	9703.8	116445	12
149	7239.0	86868	12

Day	Seqs. persample (mean)	Seqs. perday (total)	Number ofsamples
150	9387.6	103264	11
165	4255.0	8510	2
175	8794.2	35177	4
302	30976.0	30976	1
364	9989.7	89907	9

References

1. **Schloss PD**, **Schubert AM**, **Zackular JP**, **Iverson KD**, **Young VB**, **Petrosino JF**. 2012. Stabilization of the murine gut microbiome following weaning. Gut Microbes **3**:383–393. doi:10.4161/gmic.21008.

2. **Yue JC**, **Clayton MK**. 2005. A similarity measure based on species proportions. Communications in Statistics-Theory and Methods **34**:2123–2131.