

NAME OF THIS STUDY

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1 Abstract

² **Introduction**

³ **Results and Discussion**

⁴ **Scaling up.** The advantage of the dual-index approach is that a large number of samples can be
⁵ sequenced using a number of primers equal to only twice the square root of the number of samples.
⁶ To fully evaluate this approach, we resequenced the V4 region of 360 samples that were previously
⁷ described by sequencing the distal end of the V35 region on the 454 GS-FLX Titanium platform
⁸ (1). In that study, we observed a clear separation between murine fecal samples obtained from 8
⁹ C57BL/6 mice at 0 to 9 (early) and 141 to 150 (late) days after weaning, and there was significantly
¹⁰ less variation between the late samples than the early samples. In addition to the mouse fecal
¹¹ samples, we allocated 2 pairs of indices to resequence our mock community. We generated 3.9
¹² million pairs of sequence reads from the 16S rRNA gene with an average coverage of 10,752.4
¹³ pairs of reads per sample (95% of the samples had more than 2,788.9 pairs of sequences) using a
¹⁴ new collection of 8-nt indices (see the supplemental material). Although individual samples were
¹⁵ expected to have various amplification efficiencies, analysis of the number of reads per index did
¹⁶ not suggest a systematic positive or negative amplification bias that could be attributed to the
¹⁷ indices. The combined error rate for the two mock communities was 0.07% before preclustering
¹⁸ and 0.01% after ($n = 14,094$ sequences). When we used UCHIME to remove chimeras and rarefied
¹⁹ to 5,000 sequences, there was an average of 30.4 OTUs (i.e., 10.4 spurious OTUs). Similar to our
²⁰ previous results, ordination of the mouse fecal samples again showed the separation between the
²¹ early and late periods and increased stabilization with age (Fig. 4) (Mantel test coefficient, 0.81; P
²² < 0.001). These results clearly indicate that our approach can be scaled to multiplex large numbers
²³ of samples.

²⁴ **Conclusions**

²⁵ **Materials and Methods**

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

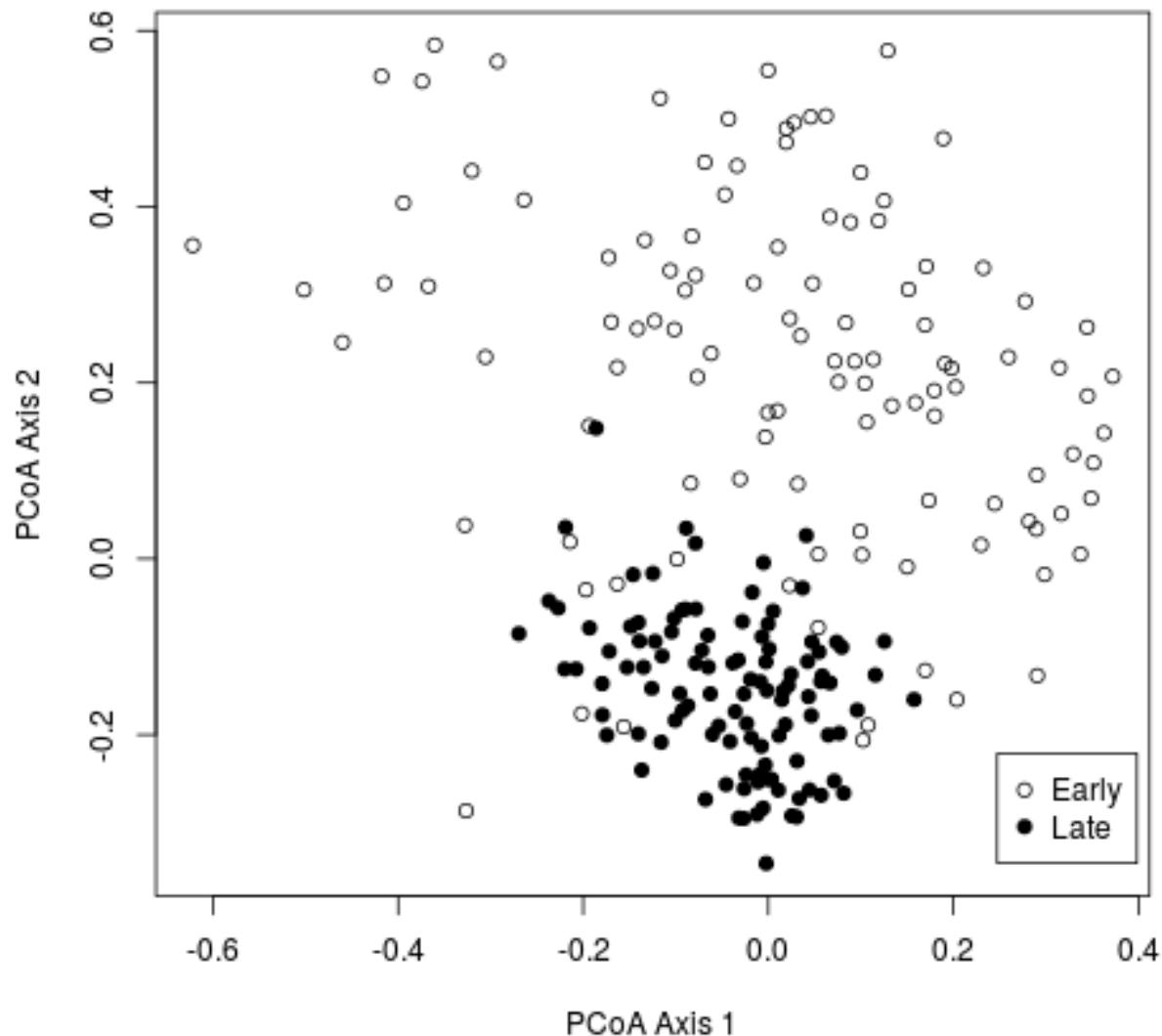


Figure 1:

29 **References**

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