Table of Contents

Supplementary Figure 1 ................................................................. S-2
Supplementary Figure 2 ................................................................. S-3
Supplementary Figure 3 ................................................................. S-4
Supplementary Figure 4 ................................................................. S-5
Supplementary Figure 5 ................................................................. S-6
Supplementary Table 1 ................................................................. S-7
Supplementary Table 2 ................................................................. S-8
Supplementary Table 3 ................................................................. S-9
**Supplementary Figure 1.** Concordance and breakdown of discordances of HiFi reads for three samples with high quality references. (A: Z. mays B73; B: Mock Metagenome C: M. musculus C57BL/6J) (a) Total concordance with the references listed in Table 1 and Supplementary Table 1. (b) mismatch discordances (c) Insertions discordances (d) deletion discordances. Boxes extend from lower to upper quartiles; the median is represented by the short line within the box; whiskers extend 1.5 interquartile distances.
Supplementary Figure 2

Supplementary Figure 2. HiFi Coverage Uniformity for the *M. musculus* C57BL/6J genome (GRCm38.p6). a) Coverage distribution levels across genomic region windows (500 bp) of varying GC content. b) Distribution of whole genome coverage in 500 bp windows. Boxes extend from lower to upper quartiles; the median is represented by the short line within the box; whiskers extend 1.5 interquartile distances.
Supplementary Figure 3

**Supplementary Figure 3.** HiFi Coverage Uniformity for the *Z. mays* B73 genome (Zm-B73-REFERENCE-NAM-5.0) a) Coverage distribution levels across genomic region windows (500 bp) of varying GC content. b) distribution of whole genome coverage in 500 bp. Boxes extend from lower to upper quartiles; the median is represented by the short line within the box; whiskers extend 1.5 interquartile distances.
**Supplementary Figure 4.** Coverage distribution for metagenome samples. (a) The 0.02% composition group. A: *S. odontolytica*, B: *E. faecalis*, C1: *D. radiodurans*-plasmid MP1, C2: *D. radiodurans*-plasmid CP1, C3: *D. radiodurans*-chromosome 1, C4: *D. radiodurans*-chromosome 2, D: *B. adolescentis*, E: *B. vulgatus*. (b) The 0.18% composition group. F1: *A. baumannii*-chromosome 1, F2: *A. baumannii*-chromosome 2, F3: *A. baumannii*-chromosome 3, F4: *A. baumannii*-chromosome 4, G: *N. meningitidis*, H: *L. gasseri*, I: *C. acnes*, J: *H. pylori*. (c) Samples comprising the 1.8% composition group. K: *S. aureus*, L: *B. cereus*, M: *C. beijerinckii*, N: *P. aeruginosa*, O: *S. agalactiae*. (d) Samples comprising the 18% composition group. P: *P. gingivalis*, Q1: *R. sphaeroides*-chromosome 1, Q2: *R. sphaeroides*-chromosome 2, R1: *S. epidermidis*-chromosome 1, R2: *S. epidermidis*-chromosome 2, R3: *S. epidermidis*-chromosome 3, R4: *S. epidermidis*-chromosome 4, R5: *S. epidermidis*-chromosome 5, R6: *S. epidermidis*-chromosome 6, R7: *S. epidermidis*-chromosome 7, S: *S. mutans*, T: *E. coli*. Boxes extend from lower to upper quartiles; the median is represented by the short line within the box; whiskers extend 1.5 interquartile distances.
Supplementary Figure 5. Sequencing coverage vs. percent composition for the mock metagenome ATCC MSA-1003. Average sequencing coverage as mapped by minmap2 using the bacterial references listed in Supplementary Table 1 is plotted against the expected relative composition of each bacterial species (percent of total).
## Supplementary Table 1

| Sample                      | Strain             | Composition (%) | Genome Reference | Genome size (Mb) |
|-----------------------------|--------------------|-----------------|------------------|------------------|
| *Acinetobacter baumannii*   | ATCC 17978         | 0.18%           | CP000521         | 3.97             |
| *Bacillus cereus*           | ATCC 10987         | 1.80%           | AE017194         | 5.22             |
| *Bacteroides vulgatus*      | ATCC 8482          | 0.02%           | CP000139         | 5.16             |
| *Bifidobacterium adolescentis* | ATCC 15703       | 0.02%           | AP009256         | 2.09             |
| *Clostridium beijerinckii*  | ATCC 35702         | 1.80%           | NZ_CP006777      | 6.00             |
| *Cutibacterium acnes*       | ATCC 11828         | 0.18%           | CP003084         | 2.49             |
| *Deinococcus radiodurans*   | ATCC BAA-816       | 0.02%           | AE000513         | 2.65             |
| *Enterococcus faecalis*     | ATCC 47077         | 0.02%           | NC_017316        | 2.74             |
| *Escherichia coli*          | ATCC 700926        | 18.0%           | U00096           | 4.64             |
| *Helicobacter pylori*       | ATCC 700392        | 0.18%           | AE000511         | 1.67             |
| *Lactobacillus gasseri*     | ATCC 33323         | 0.18%           | CP000413         | 1.89             |
| *Neisseria meningitidis*    | ATCC BAA-335       | 0.18%           | AE002098         | 2.27             |
| *Porphyromonas gingivalis*  | ATCC 33277         | 18.0%           | AP009380         | 2.35             |
| *Pseudomonas aeruginosa*    | ATCC 9027          | 1.80%           | PDLX01000000     | 6.34             |
| *Rhodobacter sphaeroides*   | ATCC 17029         | 18.0%           | CP000577         | 4.37             |
| *Schaalia odontolytica*     | ATCC 17982         | 0.02%           | NC_009050        |                 |
| *Staphylococcus aureus*     | ATCC BAA-1556      | 1.80%           | CP000255         | 2.87             |
| *Staphylococcus epidermidis*| ATCC 12228         | 18.0%           | AE015929         | 2.50             |
| *Streptococcus agalactiae*  | ATCC BAA-611       | 1.80%           | AE009948         | 2.16             |
| *Streptococcus mutans*      | ATCC 700610        | 18.0%           | AE014133         | 2.03             |

**Supplementary Table 1.** Bacterial strain composition of metagenome staggered mix (ATCC® MSA-1003™).
### Supplementary Table 2

| SRA accession  | Organism                | Number of subreads (Millions) | Total bases (Gb) |
|----------------|-------------------------|------------------------------|------------------|
| SRR12358174    | *M. musculus*           | 32.7                         | 488              |
| SRR12371718    | *M. musculus*           | 31.2                         | 466              |
| SRR12358173    | *Z. mays*               | 56.0                         | 782              |
| SRR12358171    | *F. x ananassa*         | 21.5                         | 428              |
| SRR12371721    | *R. muscosa*            | 24.4                         | 346              |
| SRR12371723    | *R. muscosa*            | 22.2                         | 318              |
| SRR12371724    | *R. muscosa*            | 29.8                         | 419              |
| SRR12371725    | *R. muscosa*            | 23.5                         | 328              |
| SRR12371726    | *R. muscosa*            | 24.5                         | 343              |
| SRR12371727    | *R. muscosa*            | 22.5                         | 316              |
| SRR12371722    | *R. muscosa*            | 22.9                         | 323              |
| SRR12358172    | *R. muscosa*            | 22.3                         | 319              |
| SRR12371719    | ATCC MSA-1003           | 57.9                         | 507              |
| SRR12358170    | ATCC MSA-1003           | 57.3                         | 512              |

**Supplementary Table 2.** Raw subread data from the 5 genome samples used for HiFi – CCS basecalling. SRA accession number, organism, number of subreads and total bases are given for each data record.
Supplementary Table 3

|           | Mouse    | Maize    | Mock Metagenome |
|-----------|----------|----------|-----------------|
| Mapped    | 99.9%    | 99.99%   | 98.9%           |
| Uniquely mapped | 93.3%    | 96.4%    | 98.4%           |
| Unmapped  | 0.1%     | 0.01%    | 1.1%            |

Supplementary Table 2. Mapping of HiFi reads to their references. Mouse, maize and mock metagenomes were mapped to their corresponding reference and categorized as mapped if the read aligned either uniquely (Mapping Quality Score of 60) or with an additional supplemental alignment (SAM Flag field bitcode of 0x800), uniquely mapped if it only aligned to one region of the genome with a Mapping Quality Score of 60, and unmapped if the read did not align to any region of the reference.