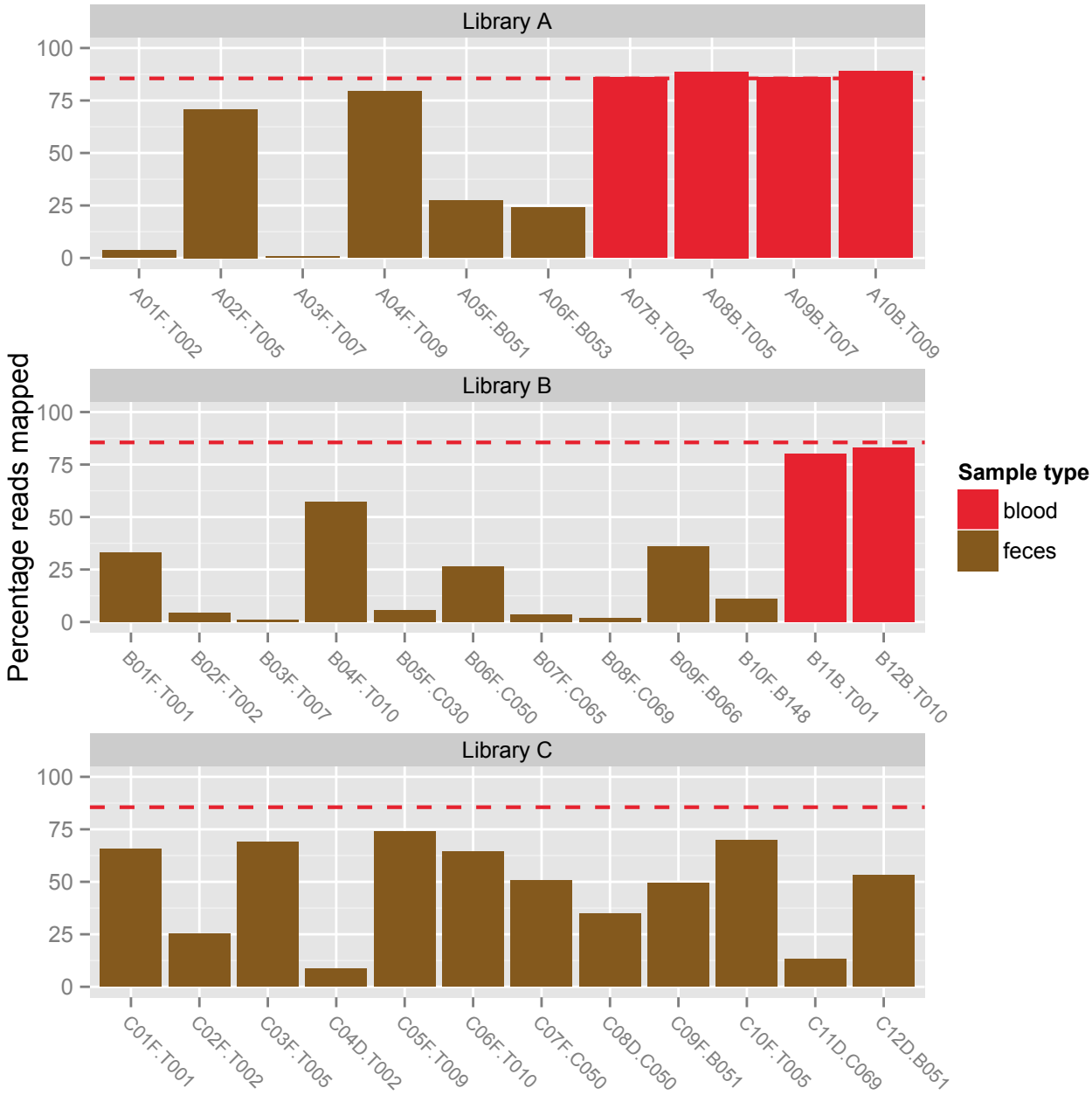
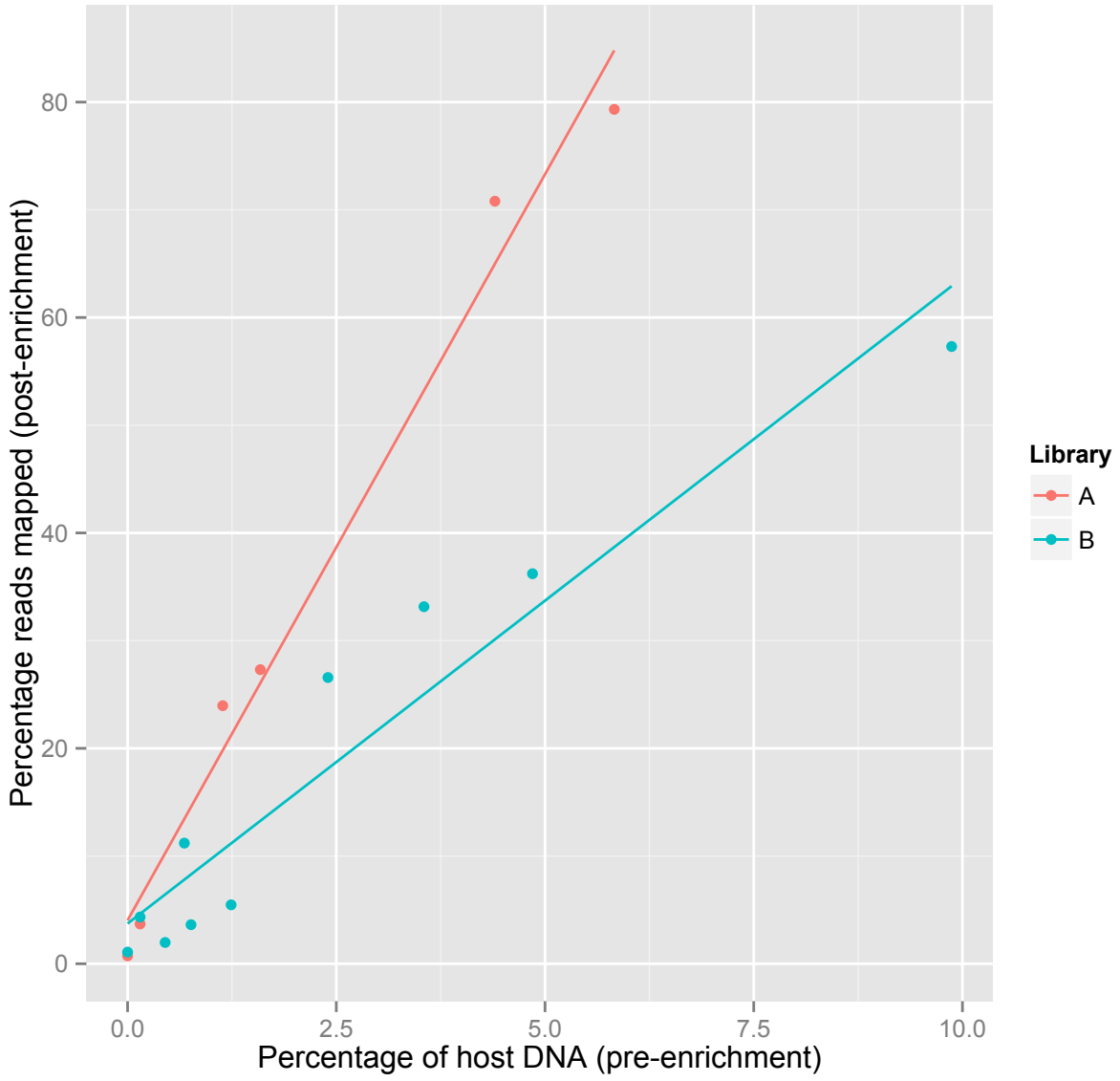


Supplementary Figure 1

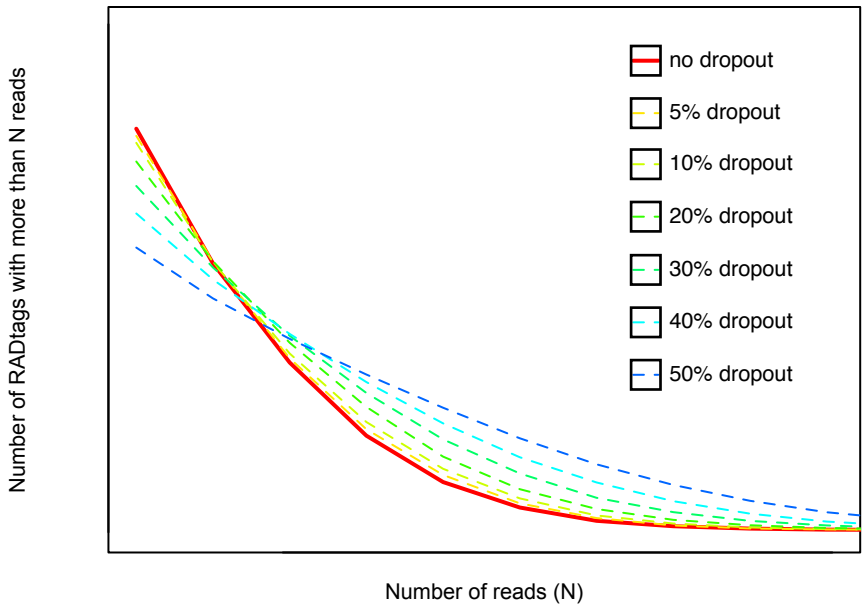
Read mapping percentages of blood and MBD-enriched fecal samples in three libraries. The dotted red horizontal line represents the mean read mapping percentage of blood samples across our study. Protocol improvements implemented following libraries A and B substantially improved enrichment in library C.



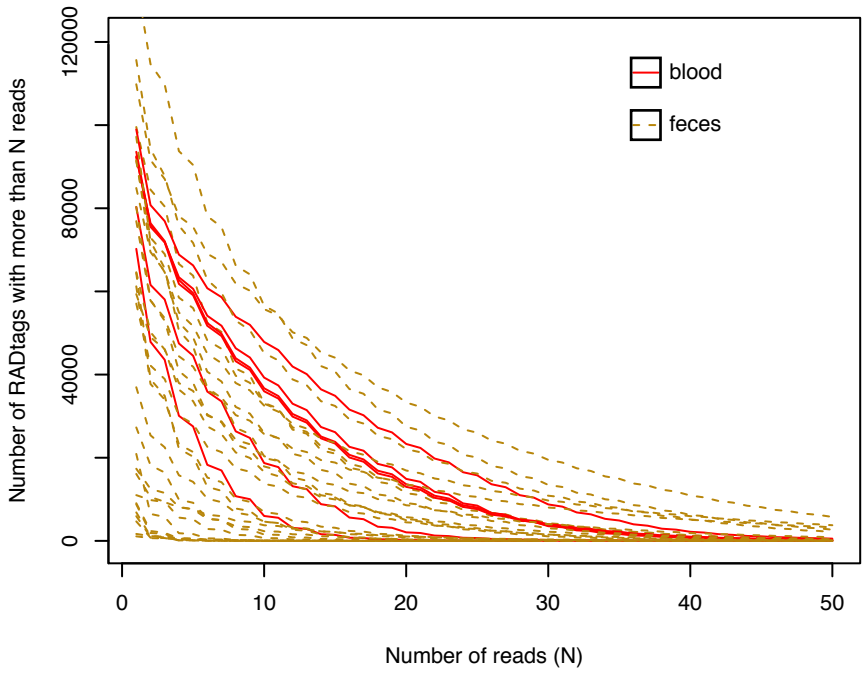
Supplementary Figure 2

Relationship between mapping percentage and pre-enrichment percentages of host DNA (libraries A and B).

a)

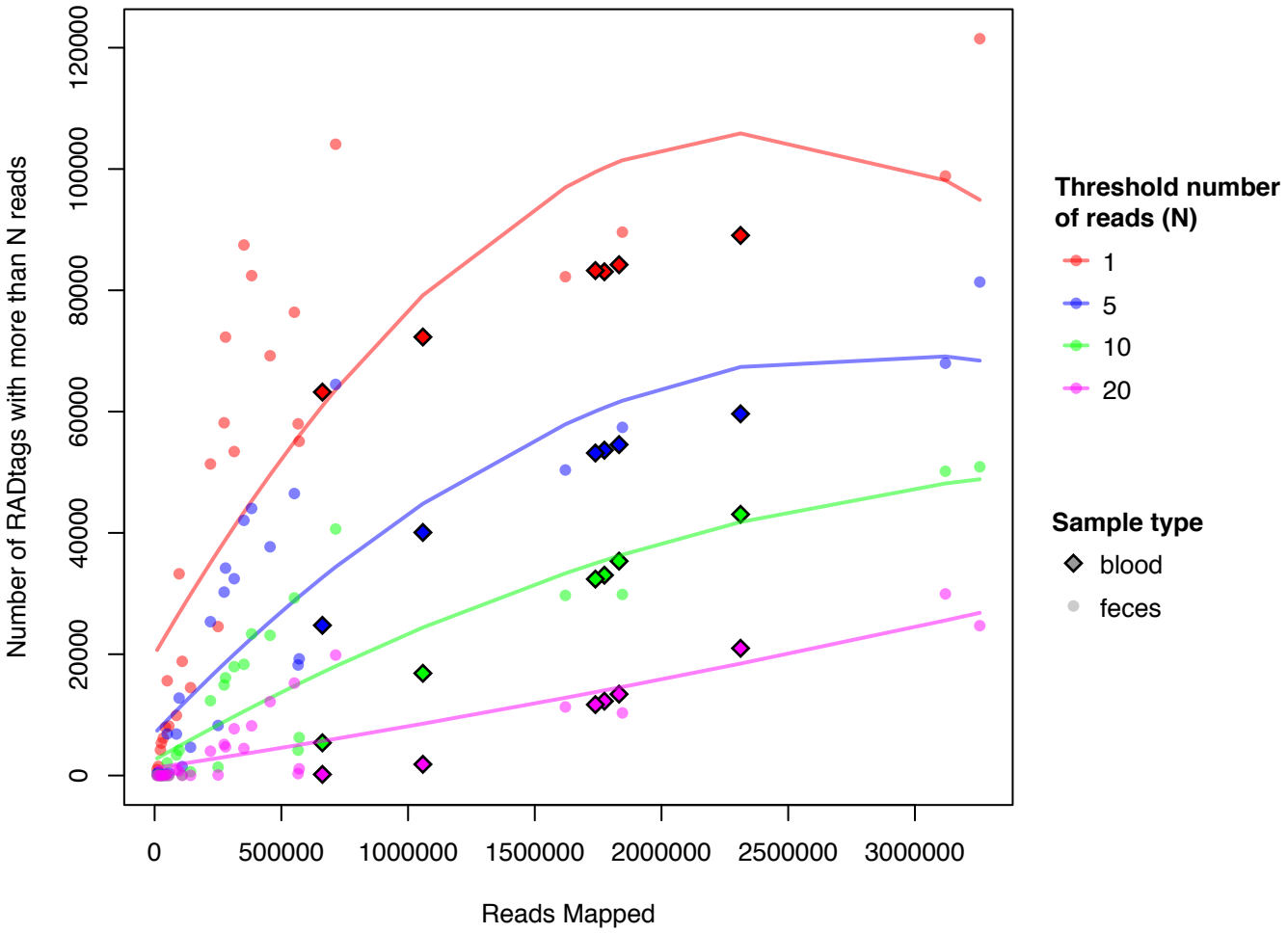


b)



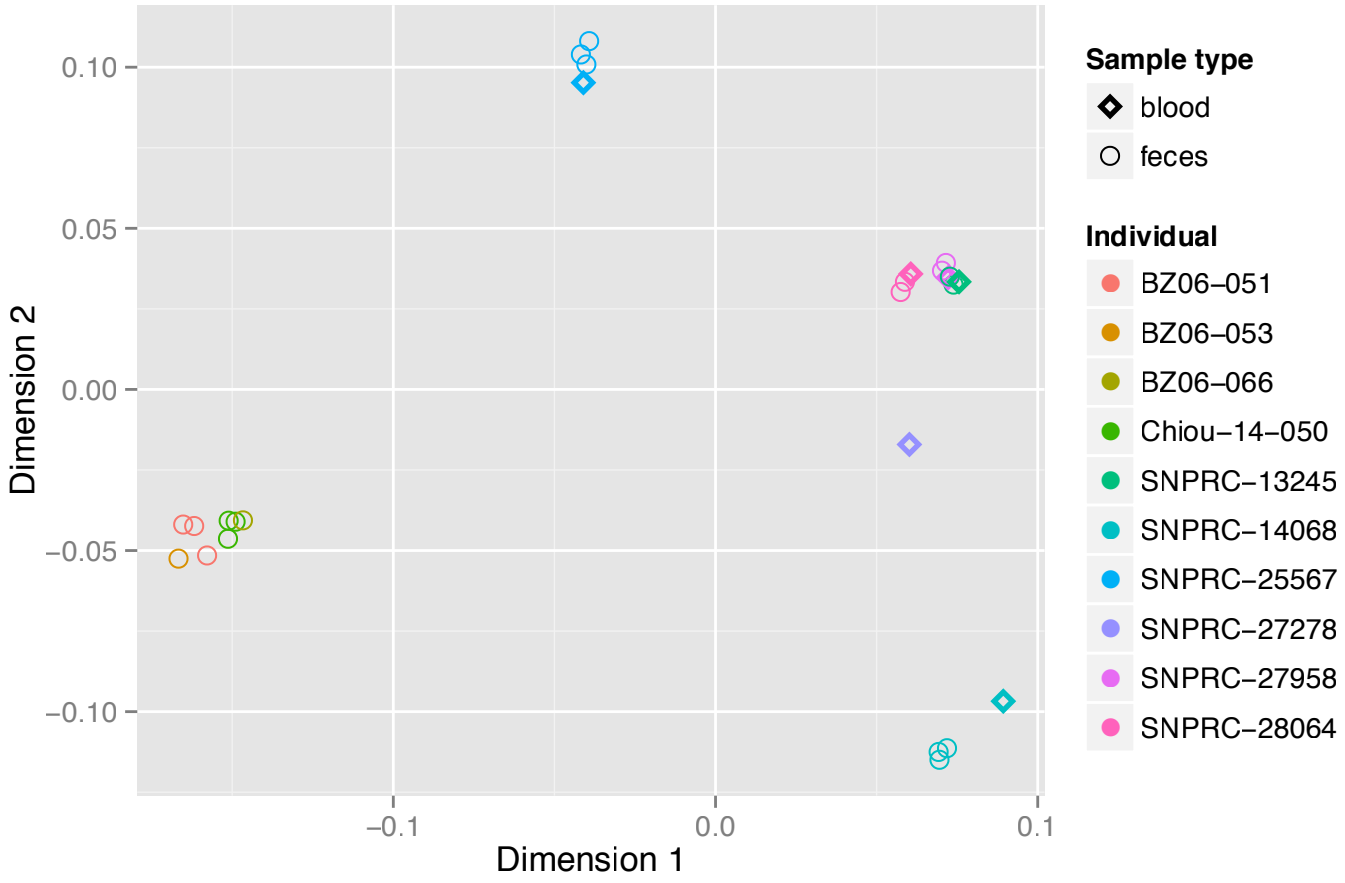
Supplementary Figure 3

Cumulative coverage curves of RADtags were (a) simulated with differing degrees of locus dropout followed by size selection to assess the effects of locus dropout on the recovery of RADtags and (b) calculated for sequenced blood- and feces-derived libraries. Based on our simulations, we expect that a dropout rate of 10% would be visually detectable in MBD-enriched fecal libraries relative to blood libraries. Cumulative coverage curves of sequenced blood- and feces-derived libraries were similar overall, indicating that a vast majority of RADtags are recovered following MBD enrichment.



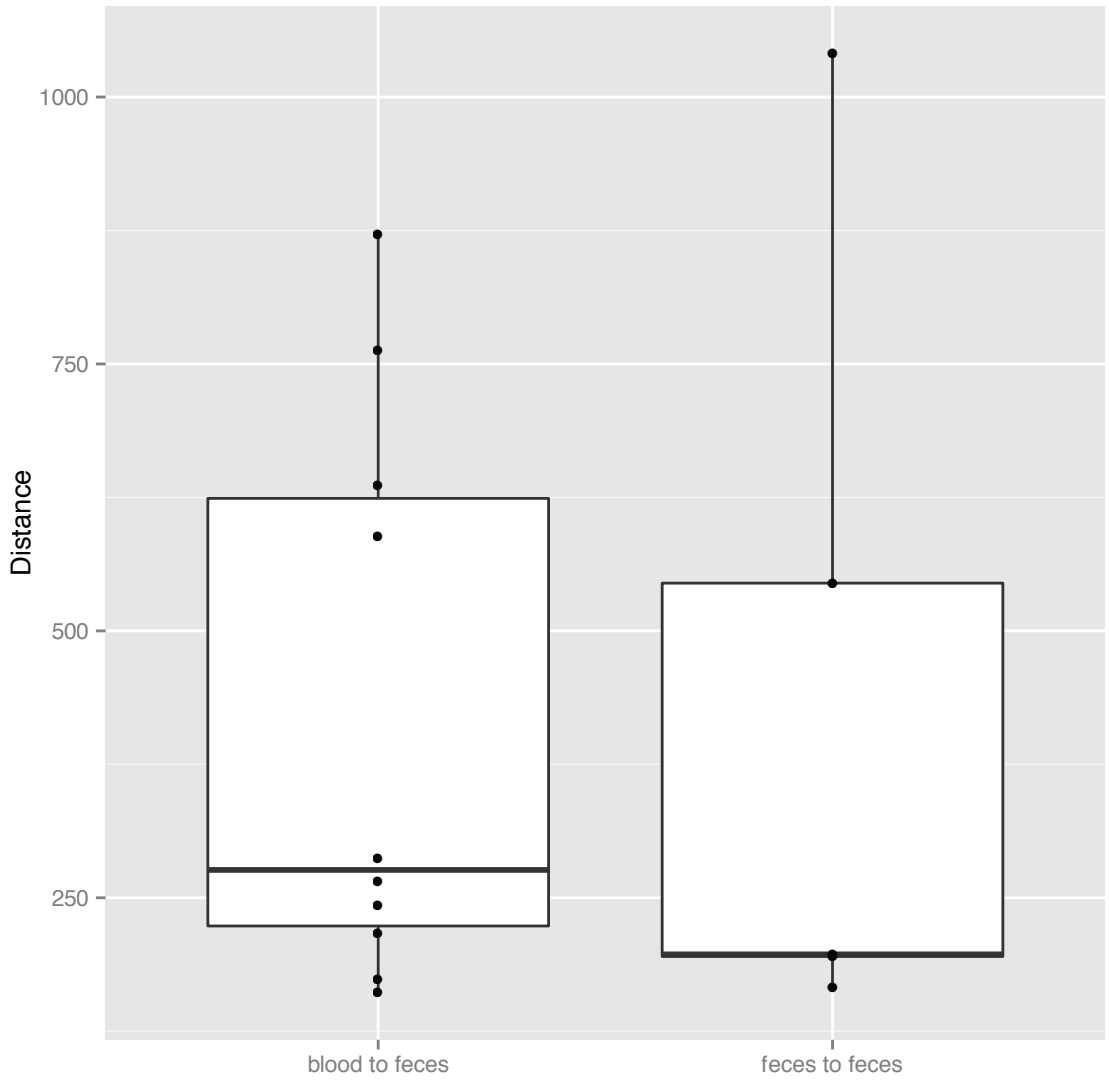
Supplementary Figure 4

RADtag recovery as a function of total number of reads mapping to the baboon reference genome. Curves are presented here using thresholds of 1, 5, 10, and 20 reads per RADtag.



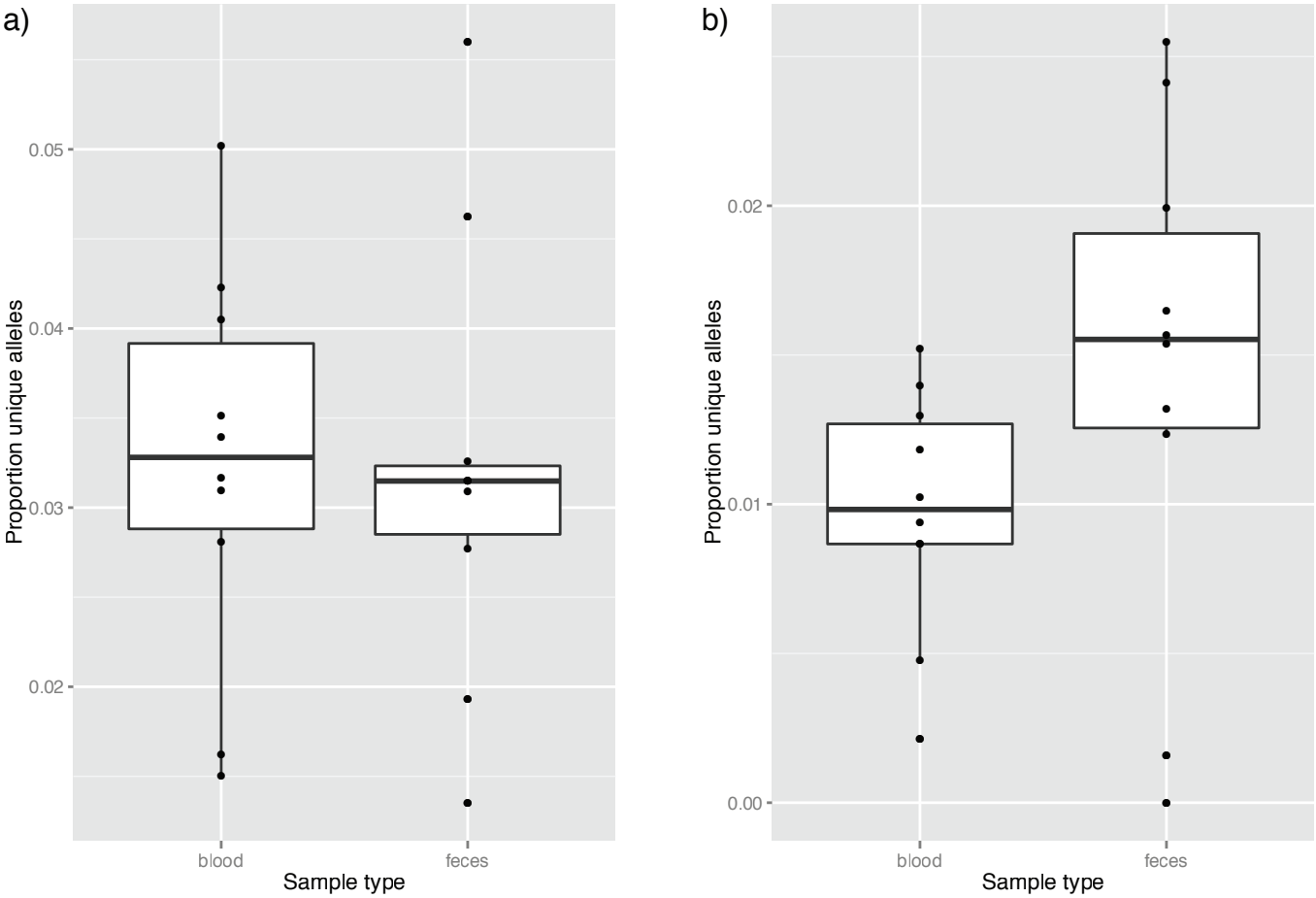
Supplementary Figure 5

Multidimensional scaling plot displaying inter-sample distances, including those between fecal and blood replicates of the same individuals.



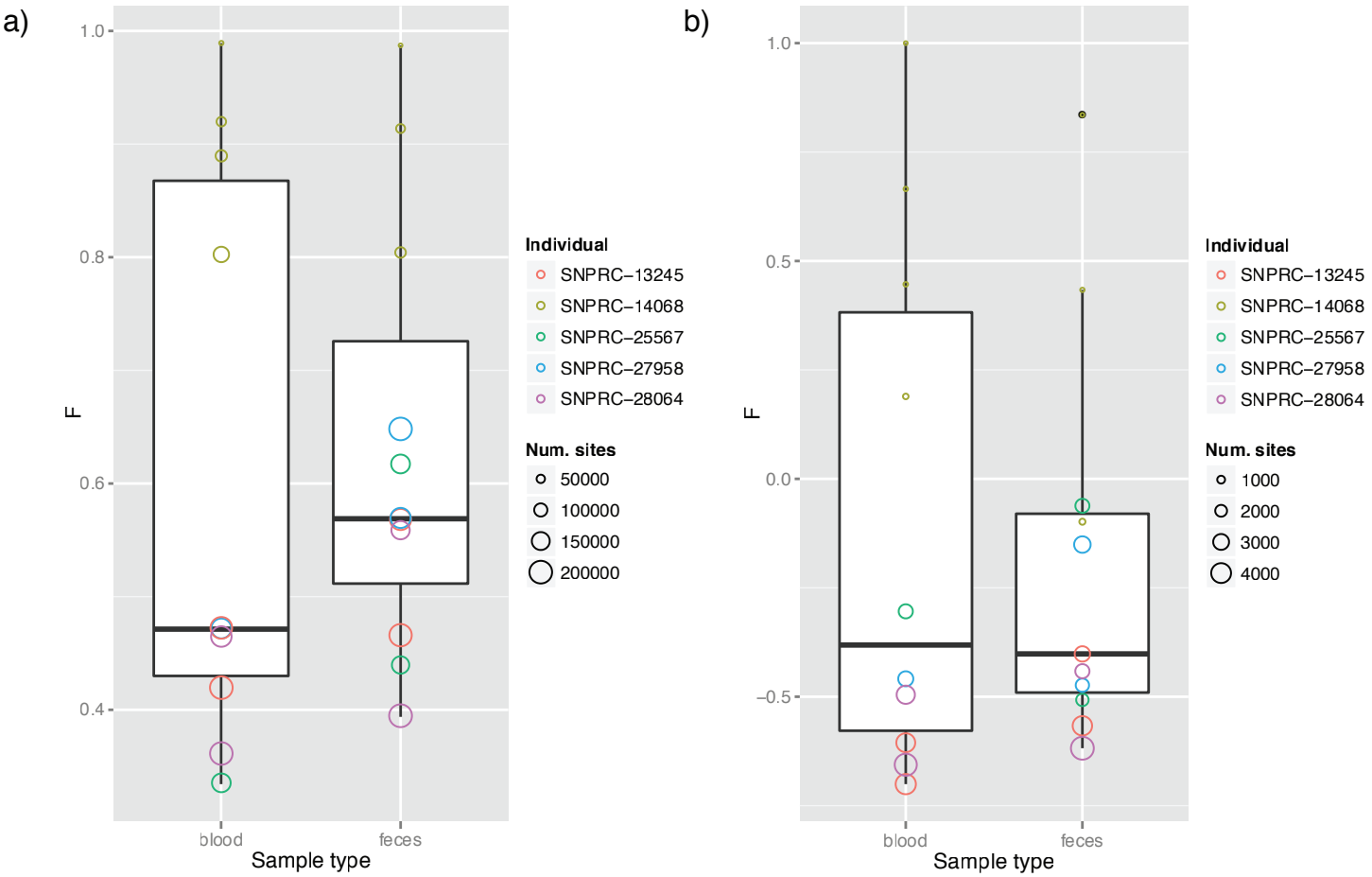
Supplementary Figure 6

Intra-individual distance comparisons for blood-feces and feces-feces replicates with equalized coverage.



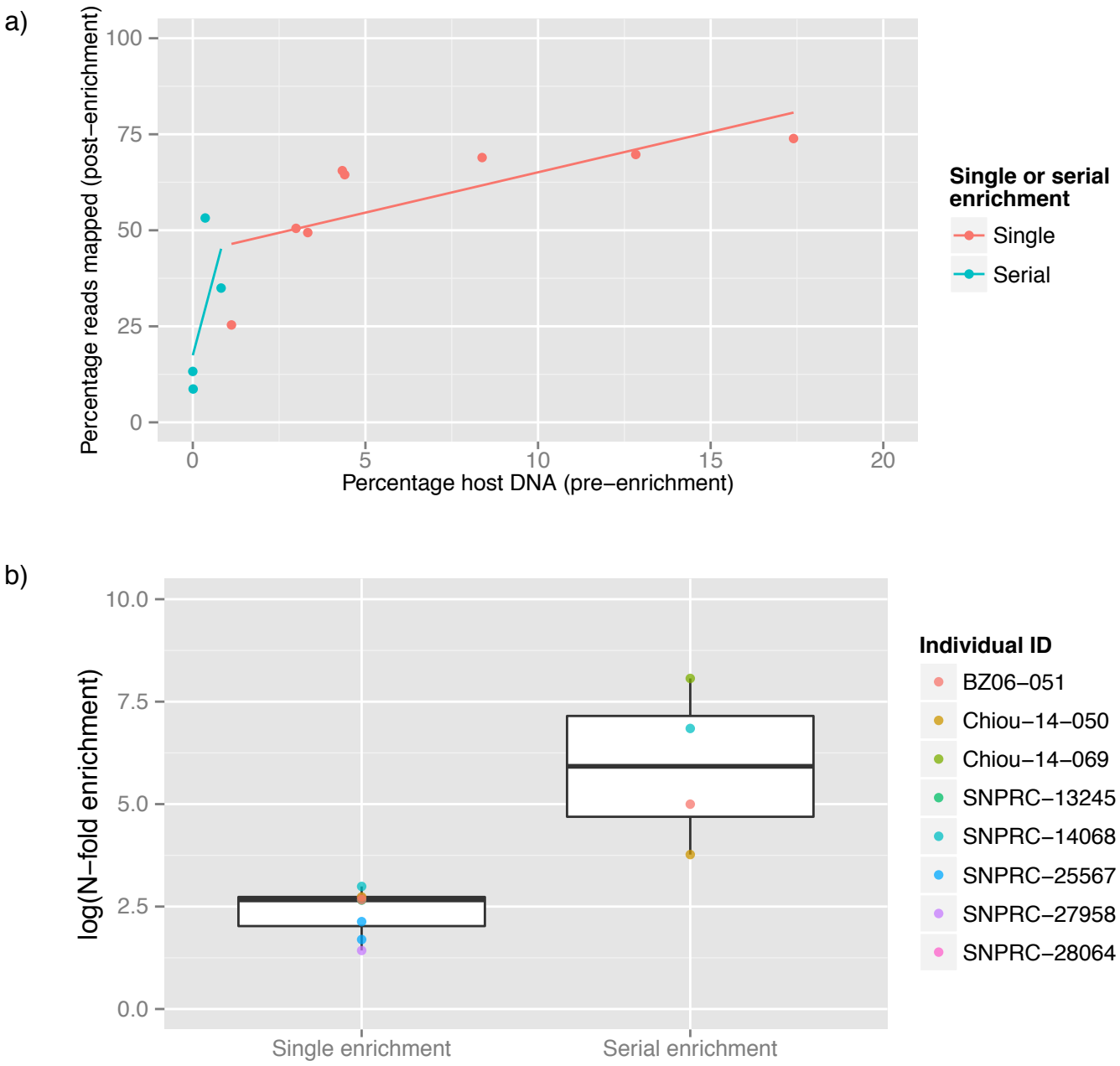
Supplementary Figure 7

Proportion of unique alleles found in blood- and feces-derived libraries with equalized coverage for (a) multi-sample-called SNPs and (b) individually called SNPs. Symmetry in the proportions of unique alleles in blood- and feces-derived libraries indicates limited dropout due to MBD enrichment.



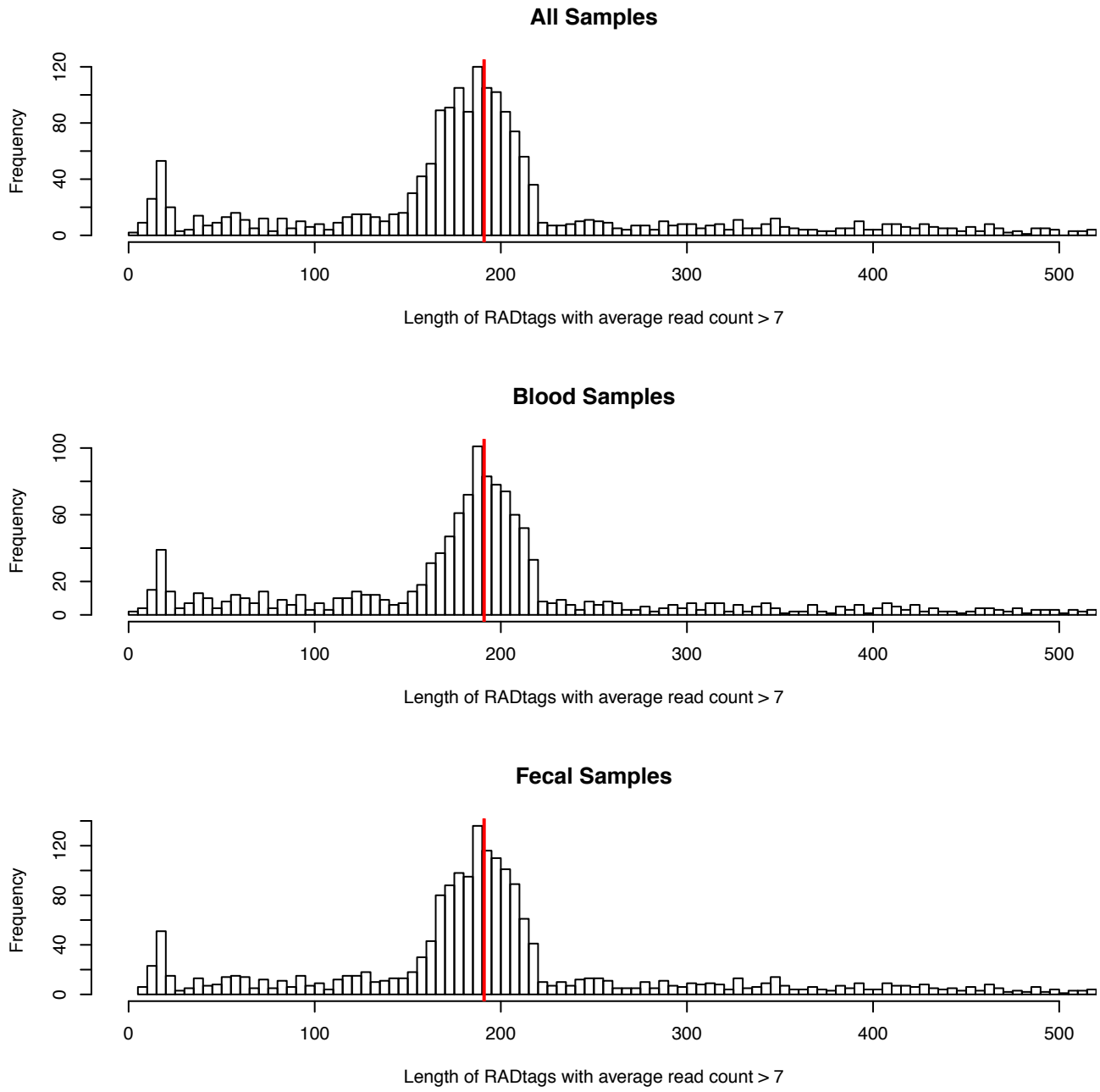
Supplementary Figure 8

Inbreeding estimates (F) from blood- and feces-derived libraries with equalized coverage for (a) multi-sample-called SNPs and (b) individually called SNPs. Note that individuals may have multiple points since the same individual may be downsampled multiple times to create pairs with equalized coverage for comparison. Homozygous sites matching the reference genome were listed as missing when variants were inferred in single individuals; therefore caution should be used when interpreting the inbreeding estimates. This, however, should not bias blood-feces comparisons.



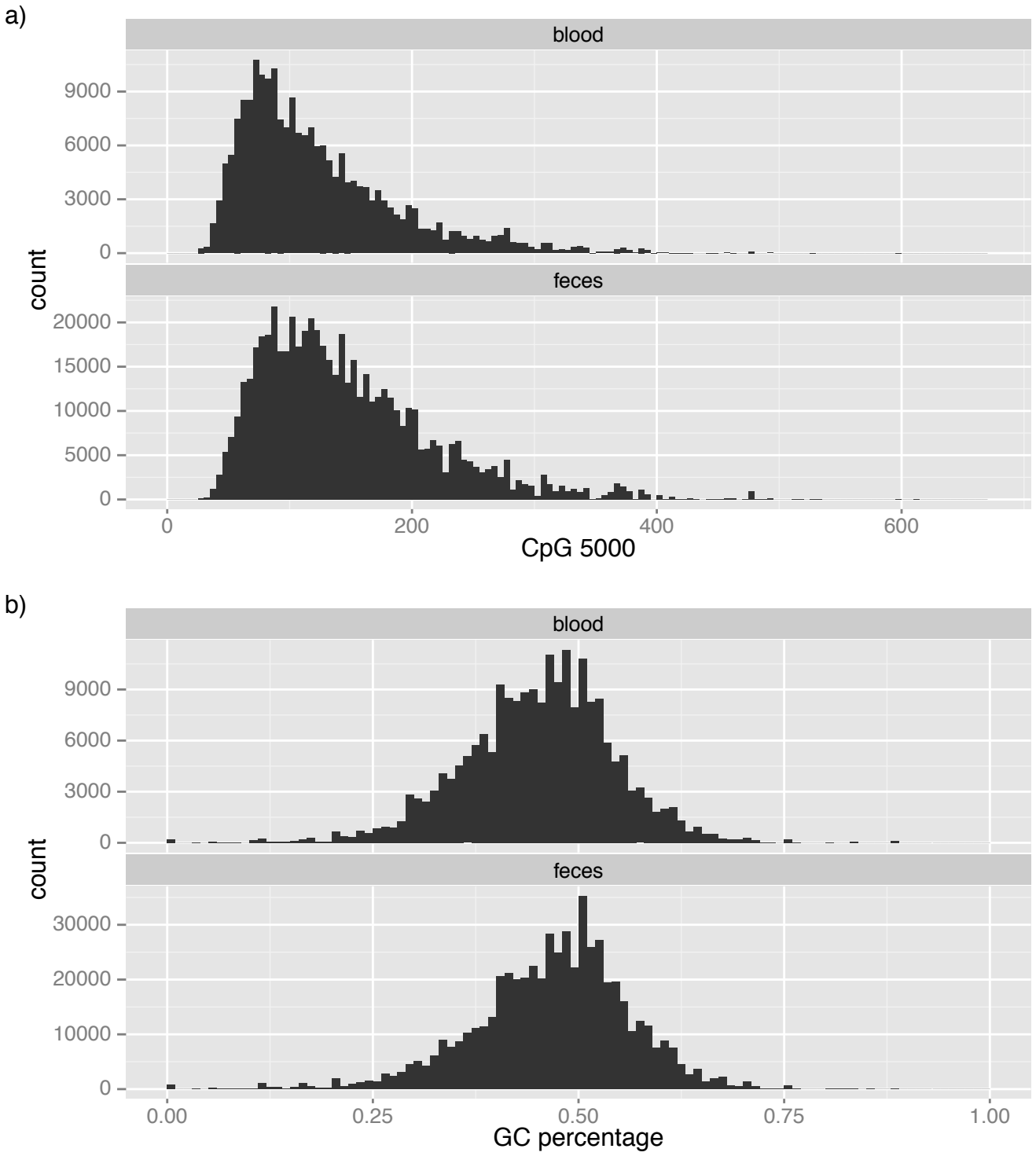
Supplementary Figure 9

A serial enrichment procedure with low-quality samples (i.e., samples with a low proportion of host DNA) (a) enriched samples with the lowest pre-enrichment host DNA proportions to sufficient levels for analysis, in many cases, and (b) resulted in higher enrichment rates relative to samples undergoing a single enrichment procedure (mean 1079-fold enrichment vs. mean 12.2-fold enrichment). Though sample size is low, the promise of serial enrichment for particularly low-quality samples is high and warrants future research.



Supplementary Figure 10

Size distribution of RADtags with at least 7 mapped reads in blood and feces-derived libraries based on a random subset of 20,000 RADtags. RADtags with lengths greater than 500bp are not shown.



Supplementary Figure 11

Distributions of (a) GC percentages and (b) local (± 5 kb) CpG densities of sequenced RADtags in blood- and feces-derived libraries.

Supplementary Note 1

In its advertised use, the Microbiome DNA Enrichment Kit (E2612S) contains enough reagents to enrich six samples, assuming 160 μl of protein A beads and 16 μl of MBD2-Fc protein are used per sample.

For FecalSeq, each reaction can be scaled down significantly. Assuming that fDNA samples on average contain 2.5% hDNA, we estimate that each reaction will require on average 4 μl of protein A beads and 0.4 μl of MBD2-Fc protein. This represents a scaling-down by a factor of 40. Therefore, a Microbiome DNA Enrichment Kit contains enough protein A beads and MBD2-Fc protein to support a total of 240 (6 * 40) enrichments.

240 enrichments at $168 / kit (university rate) = $0.70 per enrichment.