Figure S1

Library	Organism	Cell Numbers	Library Type	Kit	Kit Version
GBBOG	ZymoBIOMICS™ Microbial Community DNA Standard: D6305	100ng DNA	Shotgun	Kapa Hyper Prep Kit	N/A
CZWOH	ZymoBIOMICS™ Microbial Community Standard cells: D6300	1E8 cells	Hi-C	Phase Genomics	1
CZWON	ZymoBIOMICS™ Microbial Community Standard cells: D6300	2.8E8 cells	Hi-C	Arima/Kapa	1
GPANX	ZymoBIOMICS™ Microbial Community Standard cells: D6300	2.8E8 cells	Hi-C	Arima/Kapa	1
GPANY	ZymoBIOMICS™ Microbial Community Standard cells: D6300	1E8 cells	Hi-C	Phase Genomics	1.5
GPANZ	ZymoBIOMICS™ Microbial Community Standard cells: D6300	1E8 cells	Hi-C	Phase Genomics	1.5
GPAOA	ZymoBIOMICS™ Microbial Community Standard cells: D6300	1E8 cells	Hi-C	Phase Genomics	1.5
GPAOB	ZymoBIOMICS™ Microbial Community Standard cells: D6300	1E8 cells	Hi-C	Phase Genomics	1.5

Figure S2

Total Aligned Length of Synthetic Metagenome Bins Before MetaBAT-LR was Applied (in bp)

Reference Genomes	bin.1	bin.10	bin.11	bin.12	bin.13	bin.14	bin.15	bin.2	bin.3	bin.4	bin.5	bin.6	bin.7	bin.8	bin.9
Bacillus subtilis	-	-	-	-	-	-	-	-	1818749	-	1135237	-	890877	-	-
Cryptococcus neoformans	-	-	-	-	-	-	1927	-	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	1117655	-	-	-	-	74	1459877	-	-	-	-	-
Escherichia coli	-	-	-	-	-	4538063	-	-	-	-	-	-	-	-	-
Lactobacillus fermentum	-	266607	236079	-	-	-	-	-	-	-	-	497487	-	-	374862
Listeria monocytogenes	-	-	-	-	-	-	-	-	74	-	-	-	-	2922858	-
Pseudomonas aeruginosa	-	-	-	6689753	-	-	-	-	-	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	4712838	-	-	-	-	-	-	-	-	-
Salmonella enterica	3801292	-	-	-	-	814409	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	2636514	79	-	-	-	-	-	-

Total Aligned Length of Synthetic Metagenome Bins After MetaBAT-LR was Applied (in bp)

Reference Genomes	bin.1	bin.10.11.9.6	bin.12.4	bin.13	bin.14	bin.15	bin.2	bin.3.7.5	bin.8
Bacillus subtilis	-	-	-	-	-	-	-	3844863	-
Cryptococcus neoformans	-	-	-	-	-	8840412	-	-	-
Enterococcus faecalis	-	-	2577532	-	-	-	-	74	-
Escherichia coli	-	-	-	-	4538063	-	-	-	-
Lactobacillus fermentum	-	1375035	-	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-	-	74	2922858
Pseudomonas aeruginosa	-	-	-	6689753	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	9546496	-	-	-
Salmonella enterica	3801292	-	-	-	814955	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	2636514	79	-

Figure S3

Percent Genome Completeness
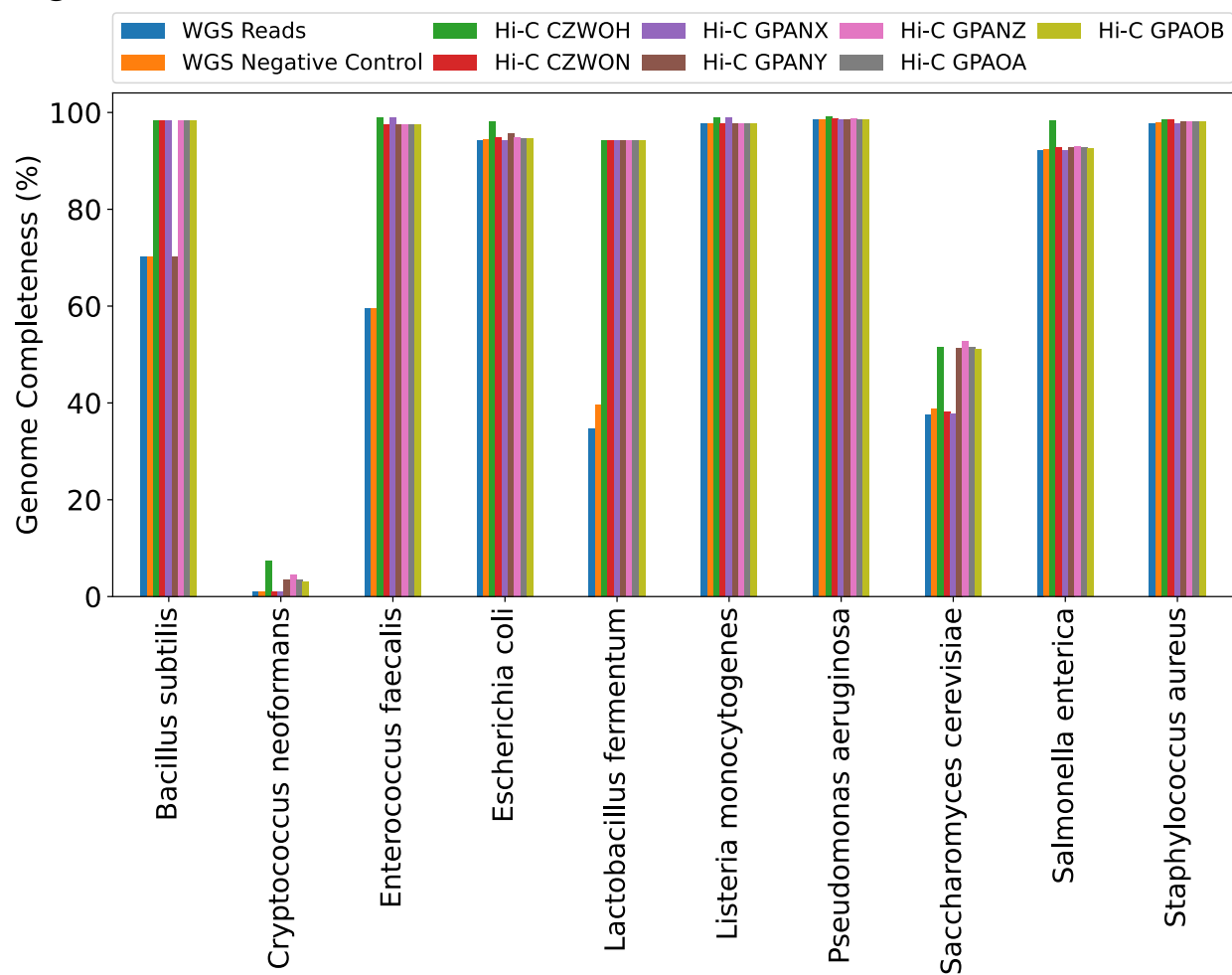in WGS with a Gradient Subsampling of Hi-C Data
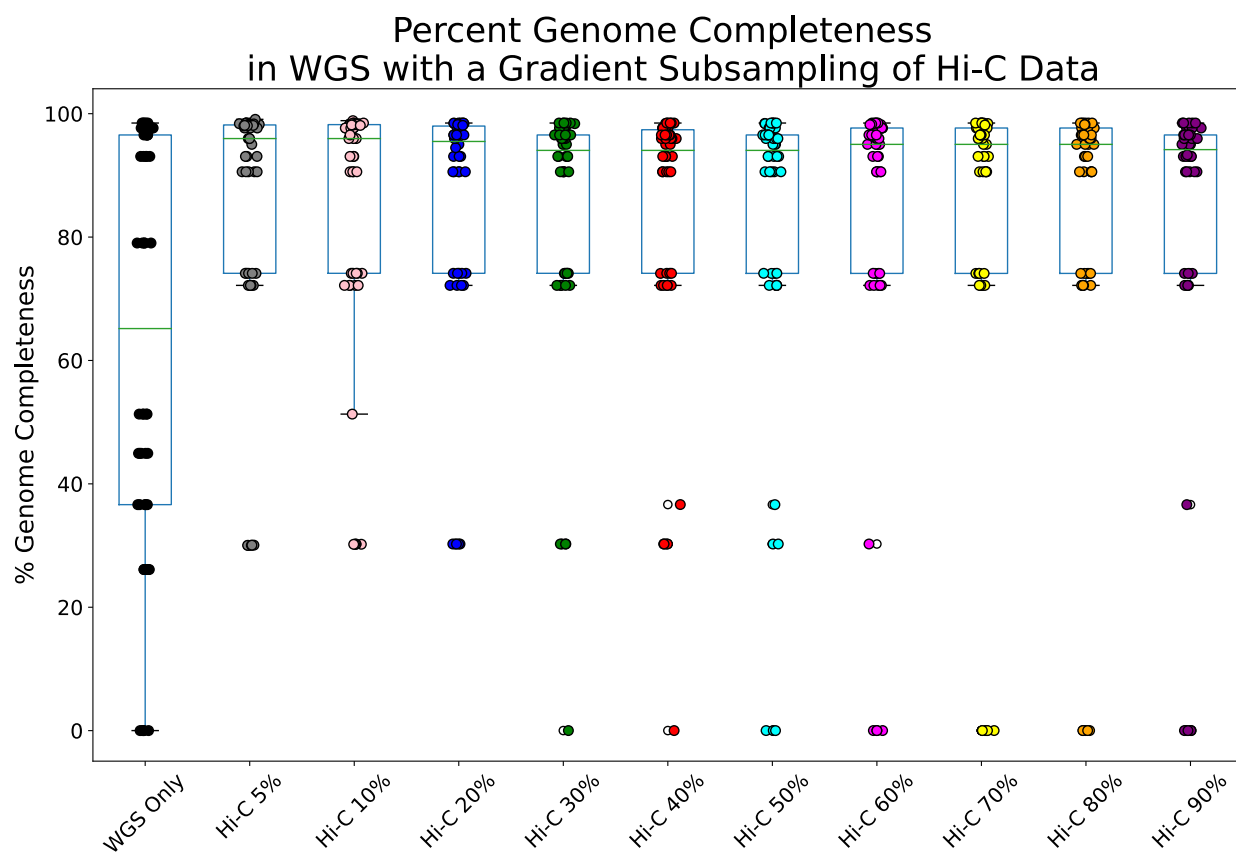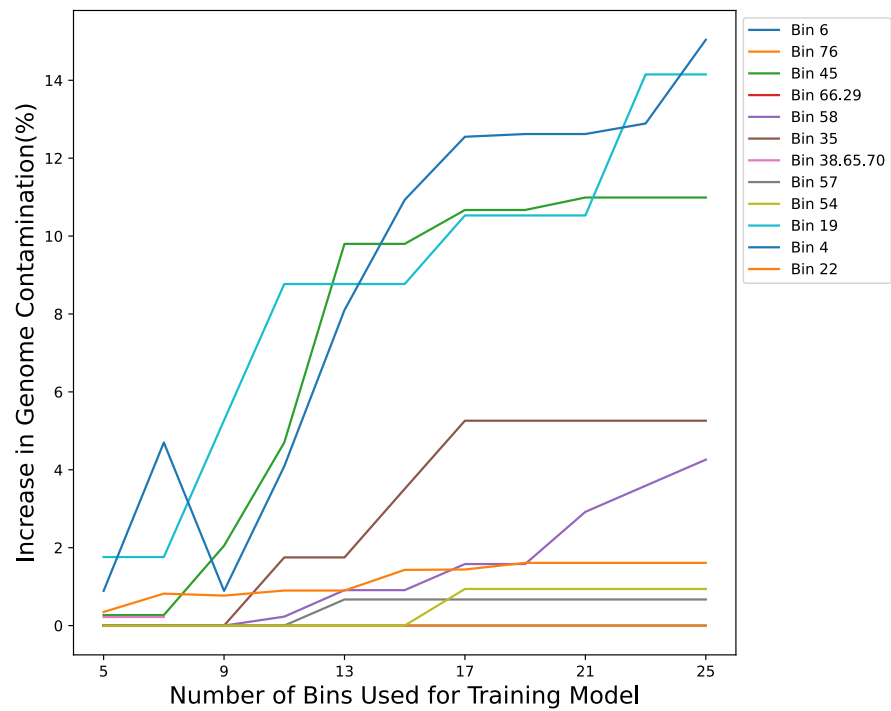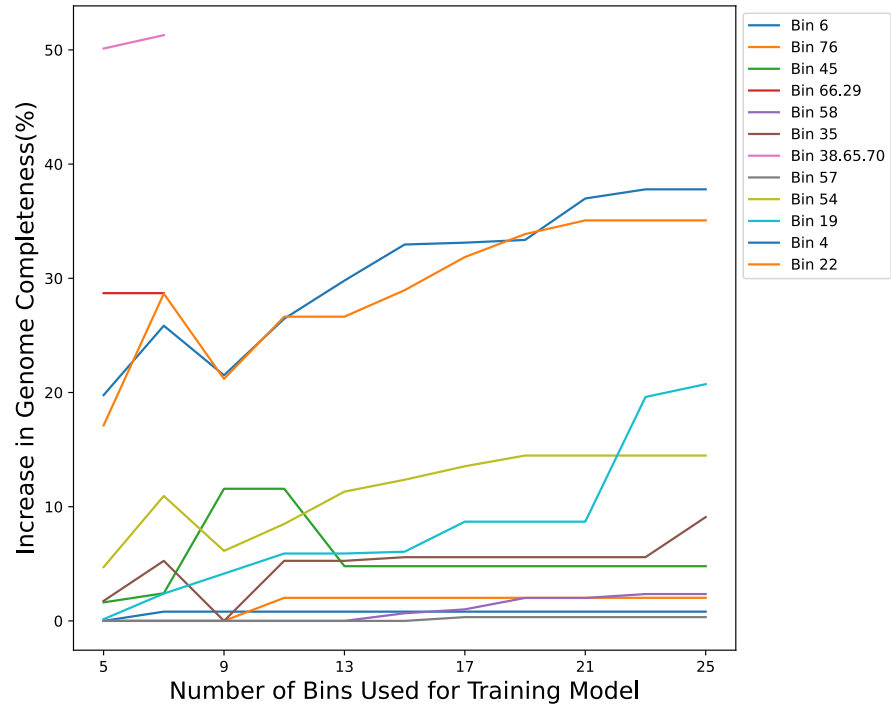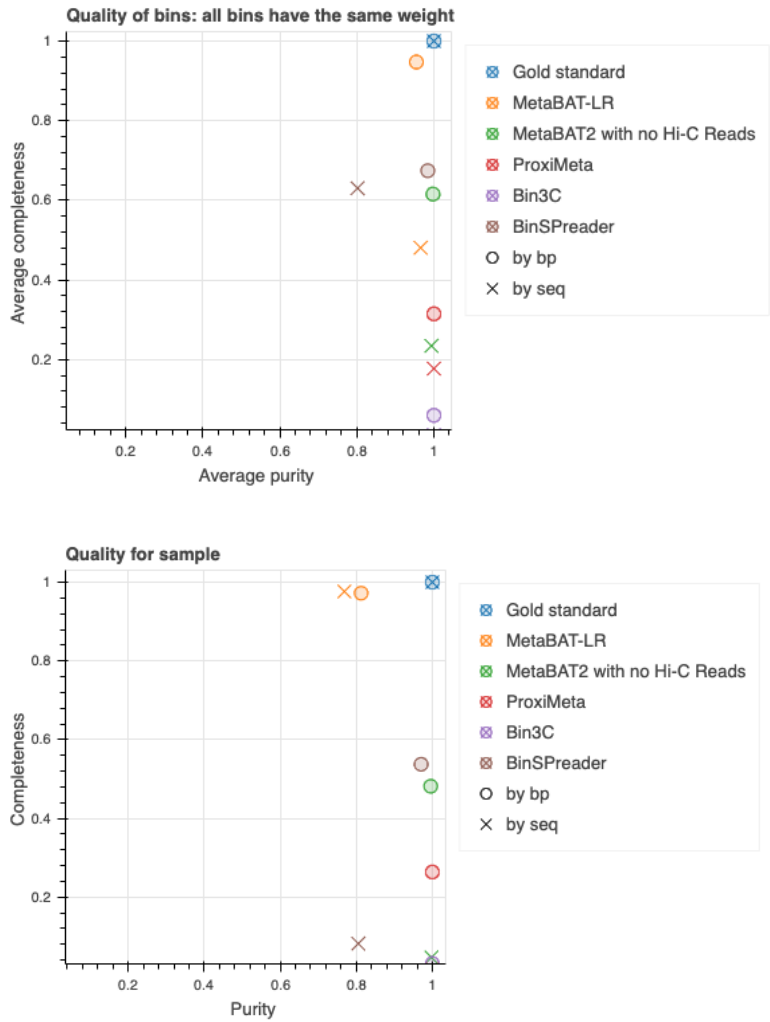
Figure S4

Figure S5

Figure S6

AMBER Metrics per Bin for Binning Results of MetaBAT-LR, Bin3C, ProxiMeta, and BinSPreader

Method	Most abundant genome	Purity (bp)	Completeness (bp)	Bin size (bp)	True positives (bp)	True size of most abundant genome (bp)
MetaBAT-LR	Cryptococcus_neoformans	0.614083	0.993729218	2.7E+07	16523956	16628228
MetaBAT-LR	Pseudomonas_aeruginosa	1	0.992840772	6689917	6689917	6738157
MetaBAT-LR	Bacillus_subtilis	1	0.973731652	3845203	3845203	3948935
MetaBAT-LR	Salmonella_enterica	1	0.959432452	3801635	3801635	3962379
MetaBAT-LR	Listeria_monocytogenes	1	0.955797301	2923826	2923826	3059044
MetaBAT-LR	Staphylococcus_aureus	1	0.952857201	2636614	2636614	2767061
MetaBAT-LR	Escherichia_coli	0.976899	0.952648595	5353282	5229614	5489552
MetaBAT-LR	Enterococcus_faecalis	1	0.933150449	2577732	2577732	2762397
MetaBAT-LR	Lactobacillus_fermentum	1	0.766190951	1375135	1375135	1794768
Bin3C	Enterococcus_faecalis	1	0.241639243	471768	471768	1952365
Bin3C	Bacillus_subtilis	1	0.235613438	1018420	1018420	4322419
Bin3C	Enterococcus_faecalis	1	0.13480676	263192	263192	1952365
Bin3C	Escherichia_coli	1	0.122800875	328637	328637	2676178
Bin3C	Enterococcus_faecalis	1	0.091916214	179454	179454	1952365
ProxiMeta	Lactobacillus_fermentum	1	0.873480603	1784375	1784375	2042833
ProxiMeta	Enterococcus_faecalis	1	0.843231533	2814066	2814066	3337240
ProxiMeta	Escherichia_coli	1	0.726853448	4419990	4419990	6080992
ProxiMeta	Bacillus_subtilis	1	0.50339061	3975773	3975773	7897988
ProxiMeta	Bacillus_subtilis	1	0.267943684	2116216	2116216	7897988
ProxiMeta	Saccharomyces_cerevisiae	1	0.201275392	2200756	2200756	10934054
BinSPreader	Pseudomonas_aeruginosa	0.943307	0.999935155	7160065	6754139	6754577
BinSPreader	Escherichia_coli	0.927832	0.999804336	5931306	5503254	5504331
BinSPreader	Staphylococcus_aureus	0.950192	0.995406724	2832163	2691099	2703517
BinSPreader	Salmonella_enterica	0.994483	0.969410795	3840621	3819432	3939952
BinSPreader	Listeria_monocytogenes	0.996269	0.960242867	2941521	2930546	3051880
BinSPreader	Enterococcus_faecalis	0.980731	0.526703106	1494751	1465948	2783253
BinSPreader	Bacillus_subtilis	0.999702	0.474988175	1894548	1893984	3987434
BinSPreader	Saccharomyces_cerevisiae	0.999599	0.466353704	5031688	5029669	10785095
BinSPreader	Enterococcus_faecalis	0.999779	0.408737186	1137870	1137619	2783253
BinSPreader	Lactobacillus_fermentum	0.999518	0.334857753	606048	605756	1808995
BinSPreader	Bacillus_subtilis	1	0.28635057	1141804	1141804	3987434

BinSPreader	Lactobacillus_fermentum	0.992778	0.249168737	454024	450745	1808995
BinSPreader	Bacillus_subtilis	0.97707	0.22361298	912567	891642	3987434
BinSPreader	Lactobacillus_fermentum	1	0.194730223	352266	352266	1808995
BinSPreader	Lactobacillus_fermentum	1	0.158831838	287326	287326	1808995

Figure S7

AMBER generated Plots for Binning Results of MetaBAT-LR, Bin3C, ProxiMeta, BinSPreader, and MetaBAT2 applied with no Hi-C Reads
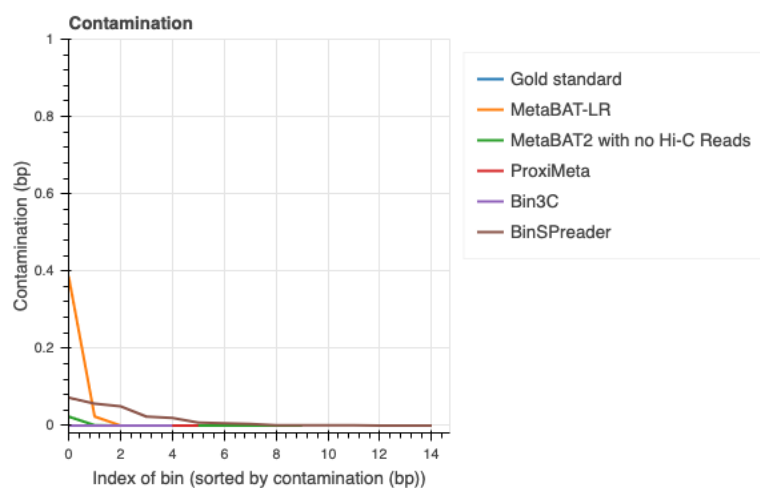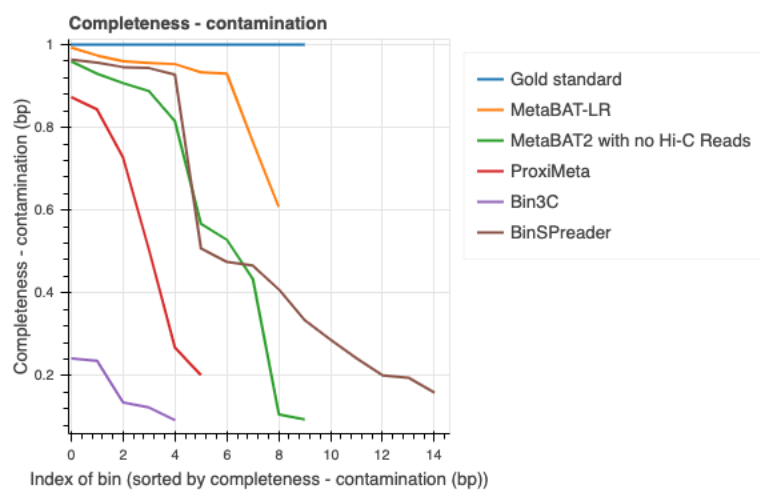
Quality per bin

- Gold standard
- MetaBAT-LR
- MetaBAT2 with no Hi-C Reads
- ProxiMeta
- Bin3C
- BinSPreader

Completeness - contamination

- Gold standard
- MetaBAT-LR
- MetaBAT2 with no Hi-C Reads
- ProxiMeta
- Bin3C
- BinSPreader

Contamination

- Gold standard
- MetaBAT-LR
- MetaBAT2 with no Hi-C Reads
- ProxiMeta
- Bin3C
- BinSPreader
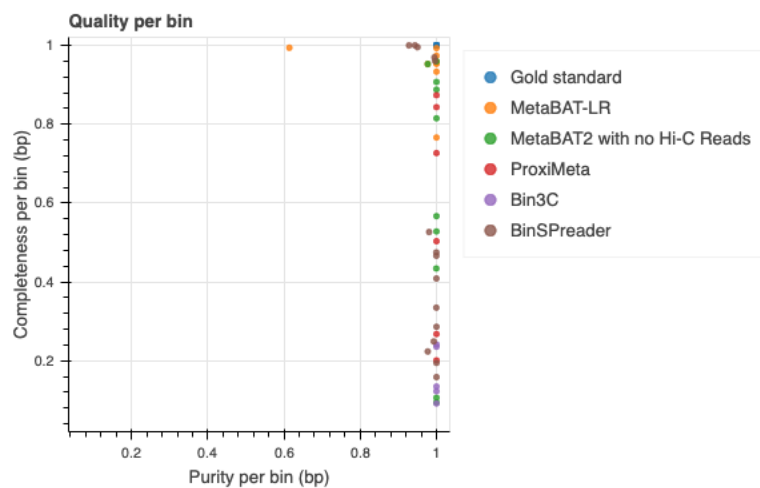
Figure S8

Metabat-LR Unique Bins from Human Fecal Dataset

Completeness	Contamination	Bin ID	Marker Lineage
96.31	0.00	bin.15.fa	o__Bacteroidales (UID2654)
89.13	4.52	Bin.49.fa	f__Lachnospiraceae (UID1256)

Figure S9

The following charts show the number of metagenome bins found by each method at varying genome completeness levels. All bins are of medium quality or higher (having genome completeness of greater than or equal to 50% and contamination levels lower than 10%)

AMBER Output Comparing Completeness for the Zymo Mock Synthetic Dataset

	Completeness				
Method	50%-60%	60%-70%	70%-80%	80%-90%	>90%
MetaBAT-LR	0	0	1	0	7
ProxiMeta	1	0	1	2	0
BinSPreader	1	0	0	0	5
Bin3C	0	0	0	0	0

dRep Output Comparing Completeness for Cat Fecal Dataset

	Completeness				
Method	50%-60%	60%-70%	70%-80%	80%-90%	>90%
MetaBAT-LR	2	3	5	7	26
ProxiMeta	1	1	7	4	28
BinSPreader	3	4	5	4	18
Bin3C	1	3	4	5	23

dRep Output Comparing Completeness for Human Fecal Dataset

	Completeness				
Method	50%-60%	60%-70%	70%-80%	80%-90%	>90%
MetaBAT-LR	2	3	5	5	23
ProxiMeta	7	0	5	3	27
BinSPreader	3	2	4	3	14
Bin3C	1	1	3	1	28