Homopolish: a method for the removal of systematic errors in nanopore sequencing by homologous polishing (Supplementary Figures S1-20)

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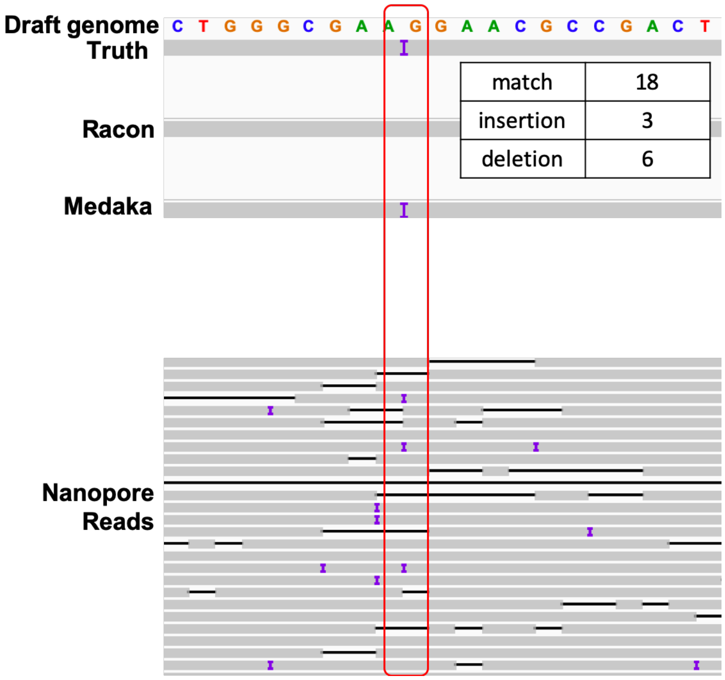


match	18
insertion	3
deletion	6

Fig. S1: Illustration of Nanopore systematic errors after polishing by Racon and by Medaka. The top shows the genomes before and after polishing. The bottom shows the IGV read alignments. Because majority of reads suggested no insertions at this locus (i.e., 18 vs 3 reads), Racon's majority-based strategy is unable to fix this error. Medaka successfully correct this systematic error by adding the missed nucleotide.

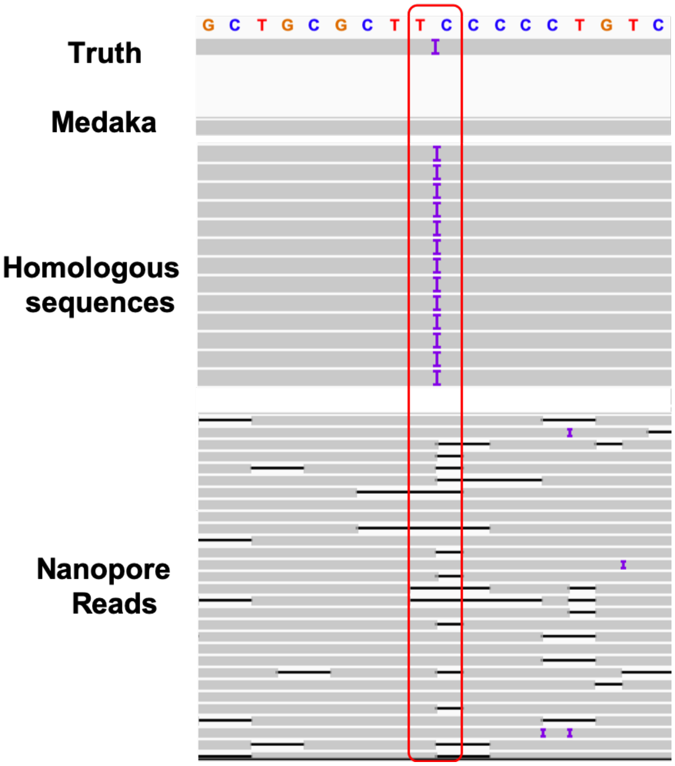


Fig. S2: Illustrations of homologous sequences for polishing Nanopore systematic errors. Because all the reads indicate no insertion at this locus (see bottom), Medaka failed to correct this sort of systematic errors. On the other hand, the homologous sequences at this region all agreed on an insertion at this locus, which in turn fix this systematic error.

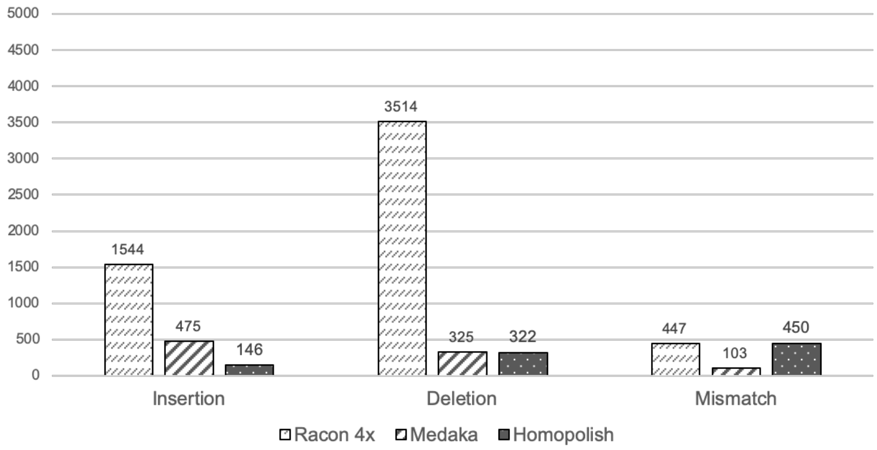


Fig. S3: Numbers of indels and mismatches of *Bacillus* genome after polishing by Racon, by Medaka, and by Homopolish.

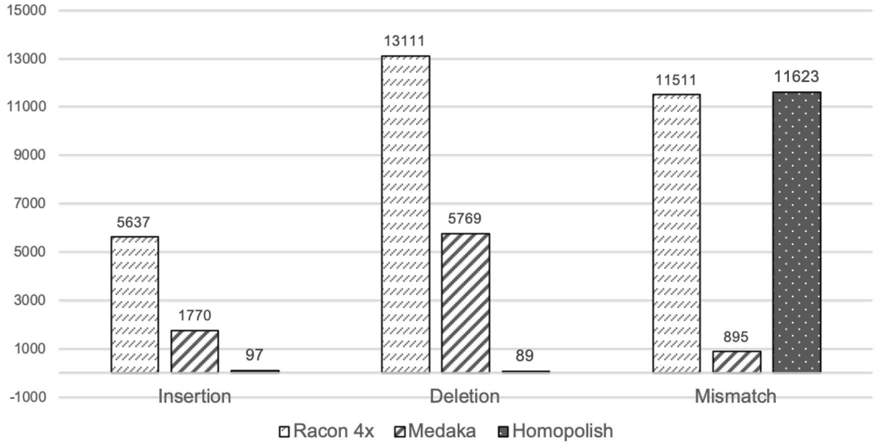
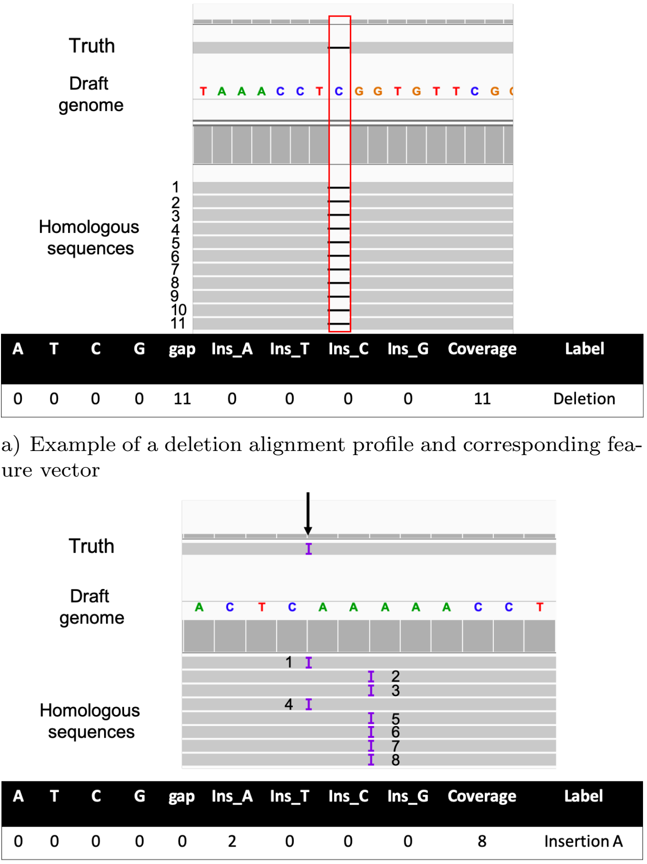


Fig. S4: Numbers of indels and mismatches of *K pneumonia* SAWA genome after polishing by Racon, by Medaka, and by Homopolish.



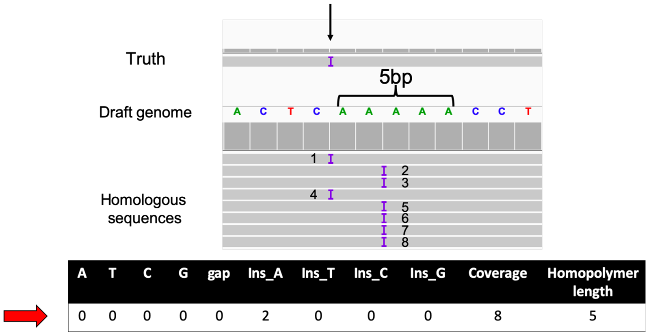
A	T	C	G	gap	Ins_A	Ins_T	Ins_C	Ins_G	Coverage	Label
0	0	0	0	11	0	0	0	0	11	Deletion

(a) Example of a deletion alignment profile and corresponding feature vector

A	T	C	G	gap	Ins_A	Ins_T	Ins_C	Ins_G	Coverage	Label
0	0	0	0	0	2	0	0	0	8	Insertion A

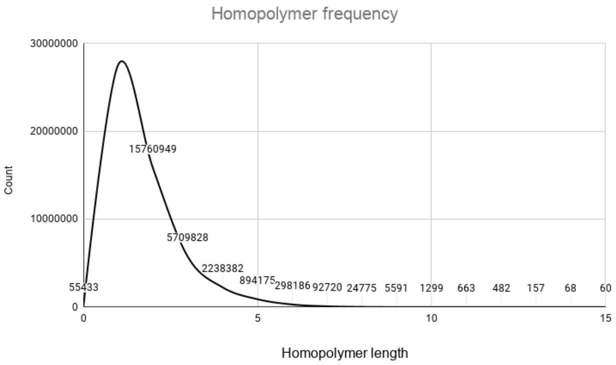
(b) Example of an insertion alignment profile and corresponding feature vector

Fig. S5: Example of feature vectors for insertions and deletions. The allele counts of each feature are shown in the corresponding count tables.



A	T	C	G	gap	Ins_A	Ins_T	Ins_C	Ins_G	Coverage	Homopolymer length
0	0	0	0	0	2	0	0	0	8	5

(a) A systematic error surrounding a homopolymer



(b) Frequency distribution of homologous lengths

Fig. S6: Illustration of Nanopore systematic errors surrounding homopolymers. (a) An insertion error at the start of a homopolymer run of five adenine bases; (b) the frequency distribution of homopolymer lengths in the genome.

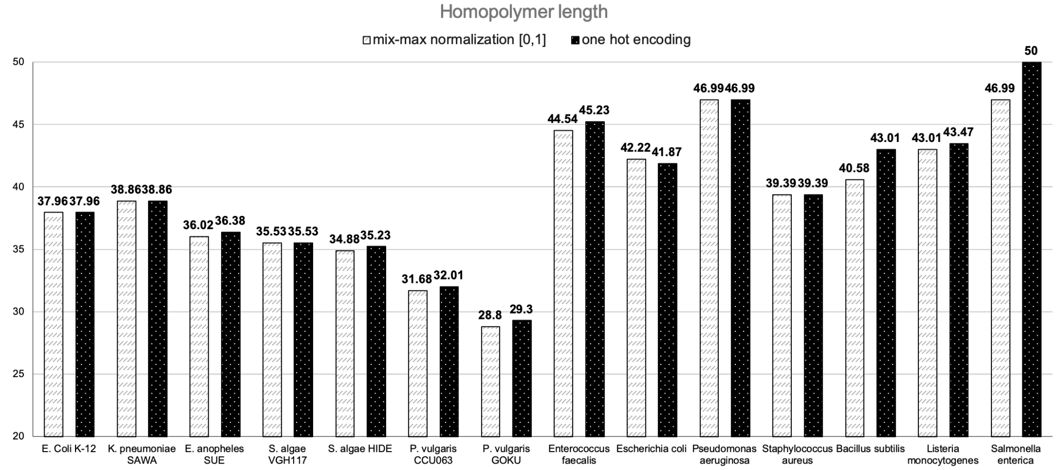


Fig. S7: Comparison of min-max normalization and one-hot encoding of the homopolymer length feature across fourteen bacteria. The accuracy is measured by the median Q scores.

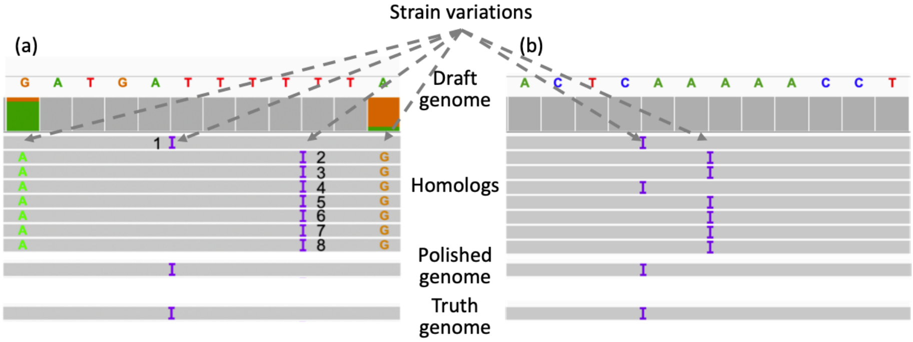
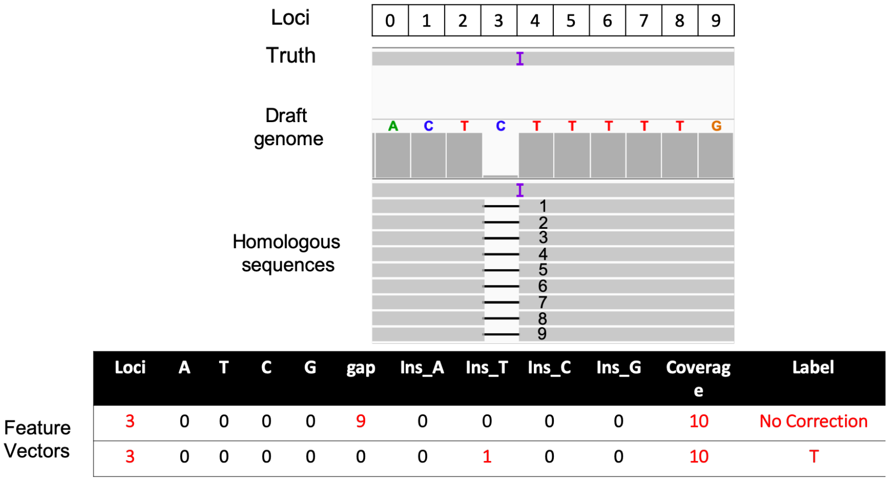
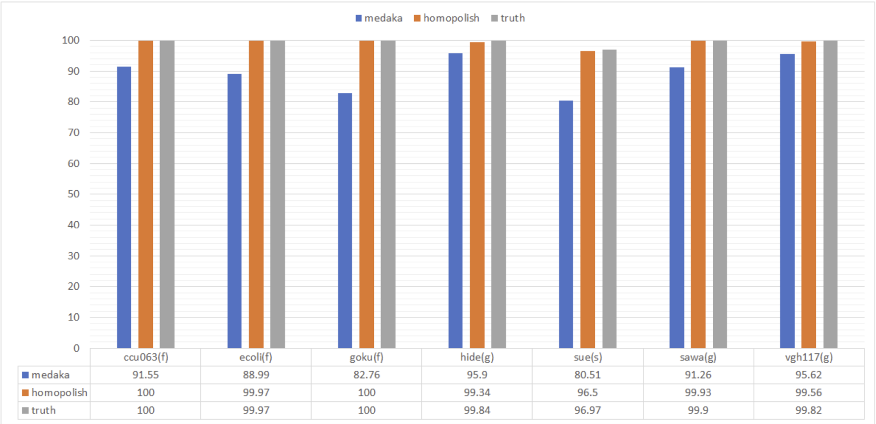


Fig. S8: Illustration of minor strain variations at multiple loci. A total of eight homologs were shown and majority of them suggest potential errors at these loci. (a) An example of distinct homologous similarity. The homologous sequence 1 flanking the minor allele is more similar to the draft genome, while those flanking the major allele contain mismatches and insertions. (b) An example of identical similarity. The homologous sequences flanking the major and the minor alleles both differ by one insertion when compared with the draft genome.



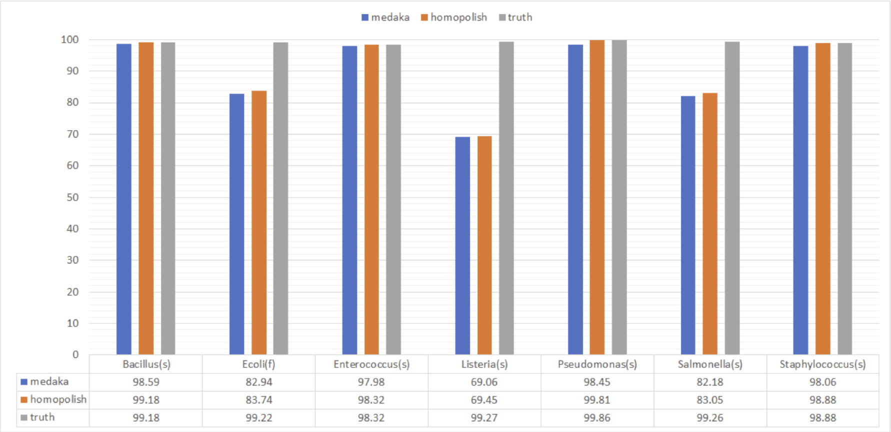
Loci	A	T	C	G	gap	Ins_A	Ins_T	Ins_C	Ins_G	Coverage	Label
3	0	0	0	0	9	0	0	0	0	10	No Correction
3	0	0	0	0	0	0	1	0	0	10	T

Fig. S9: Comparison of feature vectors of a deletion and an insertion loci. The feature vector containing the deletion alleles is distant from that containing the insertion allele.



	ccu063(f)	ecoli(f)	goku(f)	hide(g)	sue(s)	sawa(g)	vgh117(g)
■ medaka	91.55	88.99	82.76	95.9	80.51	91.26	95.62
■ homopolish	100	99.97	100	99.34	96.5	99.93	99.56
■ truth	100	99.97	100	99.84	96.97	99.9	99.82

Fig. S10: Comparison of genome completeness (CheckM) of the seven bacteria isolates.



	Bacillus(s)	Ecoli(f)	Enterococcus(s)	Listeria(s)	Pseudomonas(s)	Salmonella(s)	Staphylococcus(s)
medaka	98.59	82.94	97.98	69.06	98.45	82.18	98.06
homopolish	99.18	83.74	98.32	69.45	99.81	83.05	98.88
truth	99.18	99.22	98.32	99.27	99.86	99.26	98.88

Fig. S11: Comparison of genome completeness (CheckM) of the R9.4 Zymo metagenomic dataset.

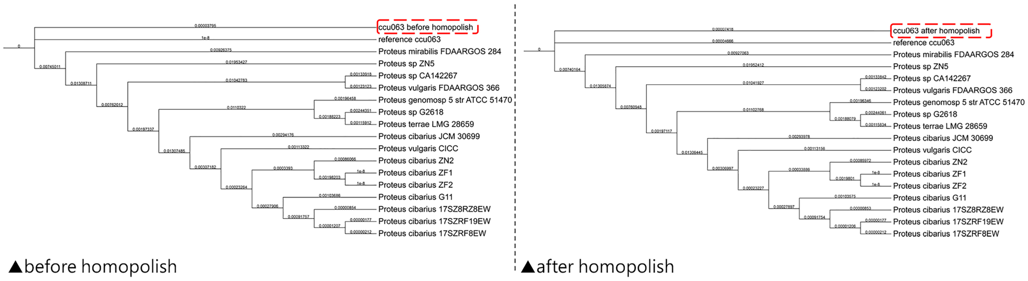


Fig. S12: Comparison of the whole-genome phylogeny of the *P vulgaris* CCU063, truth genome, and sixteen related genomes before and after Homopolish correction. The most closely-related genomes were retrieved from NCBI by Mash (>95% identity).

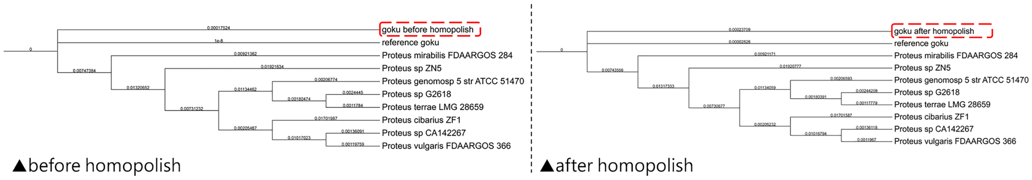


Fig. S13: Comparison of the whole-genome phylogeny of the *P vulgaris* GOKU, truth genome, and eight related genomes before and after Homopolish correction. The most closely-related genomes were retrieved from NCBI by Mash (>95% identity).

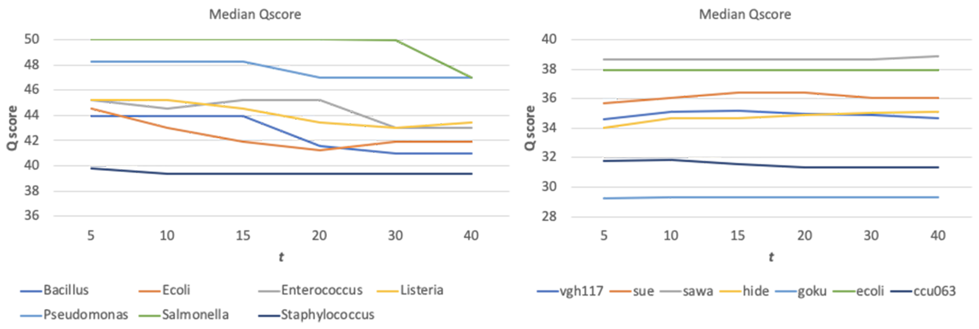


Fig. S14: The correlation of genome quality (median Q score) with respect to the maximum number of related genomes (t) retrieved by Homopolish.

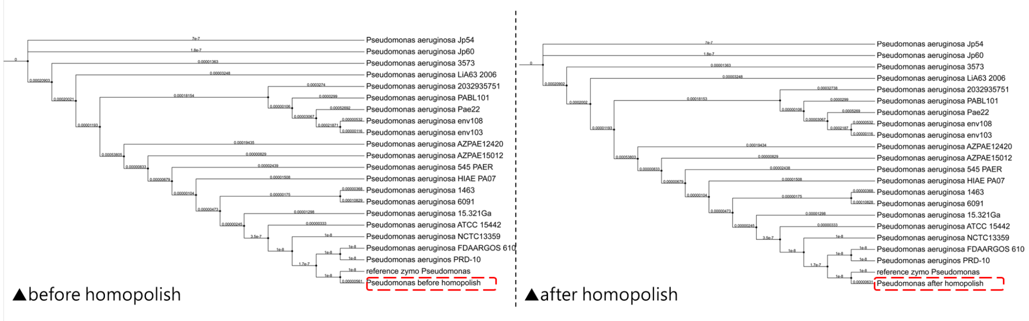

Pseudomonas aeruginosa Jp54
Pseudomonas aeruginosa Jp60
Pseudomonas aeruginosa 3573
Pseudomonas aeruginosa LiA63 2006
Pseudomonas aeruginosa 2032935751
Pseudomonas aeruginosa PABL101
Pseudomonas aeruginosa Pae22
Pseudomonas aeruginosa env108
Pseudomonas aeruginosa env103
Pseudomonas aeruginosa AZPAE12420
Pseudomonas aeruginosa AZPAE15012
Pseudomonas aeruginosa 545 PAER
Pseudomonas aeruginosa HIAE PA07
Pseudomonas aeruginosa 1463
Pseudomonas aeruginosa 6091
Pseudomonas aeruginosa 15.321Ga
Pseudomonas aeruginosa ATCC 15442
Pseudomonas aeruginosa NCTC13359
Pseudomonas aeruginosa FDAARGOS 610
Pseudomonas aeruginos PRD-10
reference zymo Pseudomonas
Pseudomonas before homopolish
▲before homopolish
Pseudomonas aeruginosa Jp54
Pseudomonas aeruginosa Jp60
Pseudomonas aeruginosa 3573
Pseudomonas aeruginosa LiA63 2006
Pseudomonas aeruginosa 2032935751
Pseudomonas aeruginosa PABL101
Pseudomonas aeruginosa Pae22
Pseudomonas aeruginosa env108
Pseudomonas aeruginosa env103
Pseudomonas aeruginosa AZPAE12420
Pseudomonas aeruginosa AZPAE15012
Pseudomonas aeruginosa 545 PAER
Pseudomonas aeruginosa HIAE PA07
Pseudomonas aeruginosa 1463
Pseudomonas aeruginosa 6091
Pseudomonas aeruginosa 15.321Ga
Pseudomonas aeruginosa ATCC 15442
Pseudomonas aeruginosa NCTC13359
Pseudomonas aeruginosa FDAARGOS 610
Pseudomonas aeruginos PRD-10
reference zymo Pseudomonas
Pseudomonas after homopolish
▲after homopolish

Fig. S15: Comparison of whole-genome phylogeny of *P aeruginosa*, its truth genome, and most closely-related genomes before and after running Homopolish. The top twenty similar genomes were retrieved from NCBI by Mash (>95% identity).

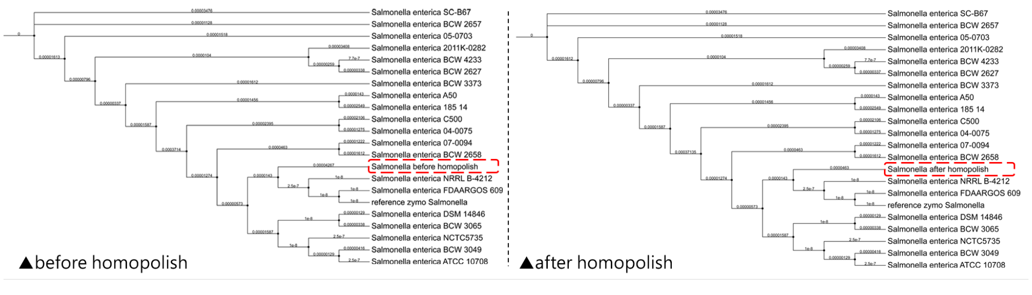

Salmonella enterica SC-B67
Salmonella enterica BCW 2657
Salmonella enterica 05-0703
Salmonella enterica 2011K-0282
Salmonella enterica BCW 4233
Salmonella enterica BCW 2627
Salmonella enterica BCW 3373
Salmonella enterica A50
Salmonella enterica 185 14
Salmonella enterica C500
Salmonella enterica 04-0075
Salmonella enterica 07-0094
Salmonella enterica BCW 2658
Salmonella before homopolish
Salmonella enterica NRRL B-4212
Salmonella enterica FDAARGOS 609
reference zymo Salmonella
Salmonella enterica DSM 14846
Salmonella enterica BCW 3065
Salmonella enterica NCTC5735
Salmonella enterica BCW 3049
Salmonella enterica ATCC 10708
▲before homopolish
Salmonella enterica SC-B67
Salmonella enterica BCW 2657
Salmonella enterica 05-0703
Salmonella enterica 2011K-0282
Salmonella enterica BCW 4233
Salmonella enterica BCW 2627
Salmonella enterica BCW 3373
Salmonella enterica A50
Salmonella enterica 185 14
Salmonella enterica C500
Salmonella enterica 04-0075
Salmonella enterica 07-0094
Salmonella enterica BCW 2658
Salmonella after homopolish
Salmonella enterica NRRL B-4212
Salmonella enterica FDAARGOS 609
reference zymo Salmonella
Salmonella enterica DSM 14846
Salmonella enterica BCW 3065
Salmonella enterica NCTC5735
Salmonella enterica BCW 3049
Salmonella enterica ATCC 10708
▲after homopolish

Fig. S16: Comparison of whole-genome phylogeny of *S enterica*, its truth genome, and most closely-related genomes before and after running Homopolish. The top twenty similar genomes were retrieved from NCBI by Mash (>95% identity).

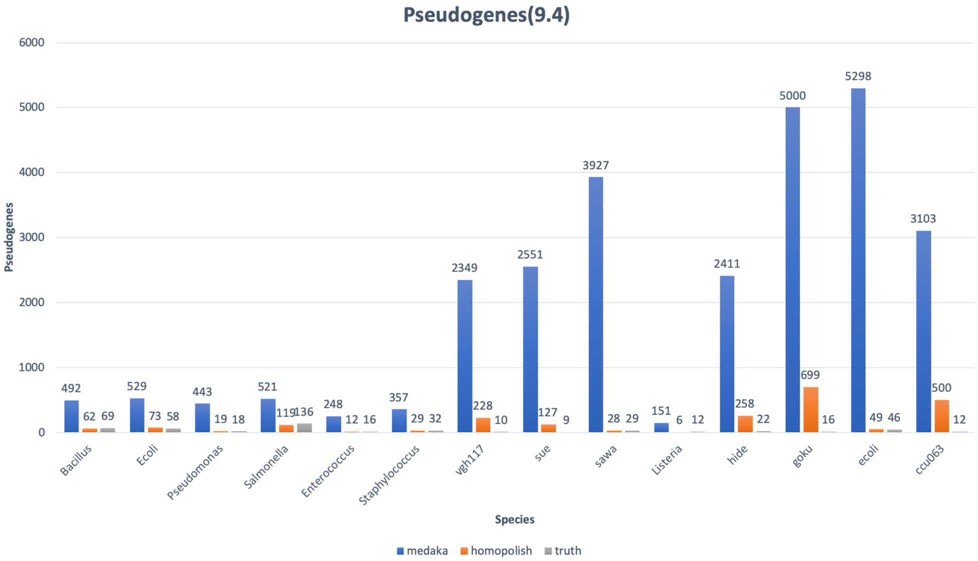


Fig. S17: Comparison of the numbers of pseudogenes in the genomes polished by Medaka, Homopolish, and the truth genomes in the Zymo metagenomic R9.4 dataset. The pseudogenes were annotated by DFAST.

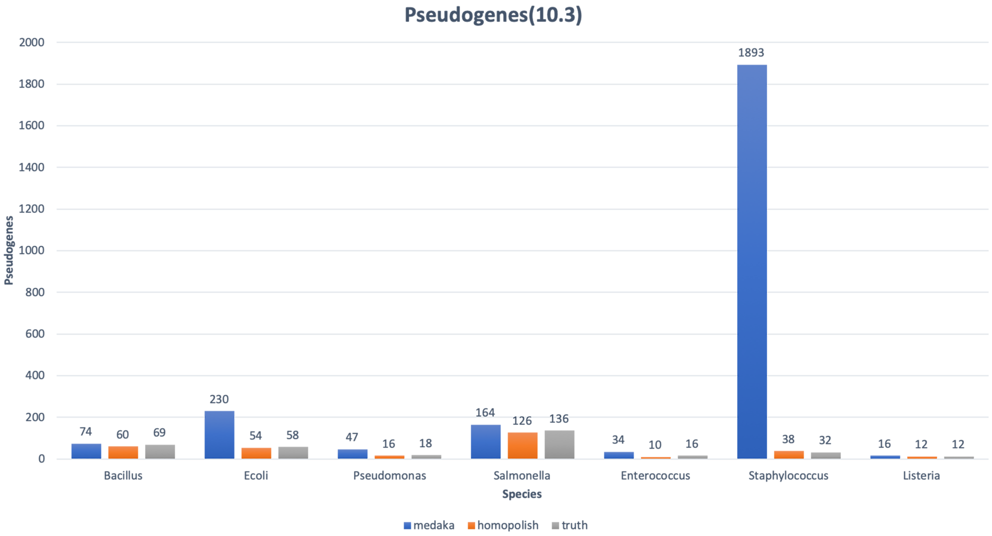


Fig. S18: Comparison of the numbers of pseudogenes in the genomes polished by Medaka, Homopolish, and the truth genomes in the Zymo metagenomic R10.3 dataset. The pseudogenes were annotated by DFAST.

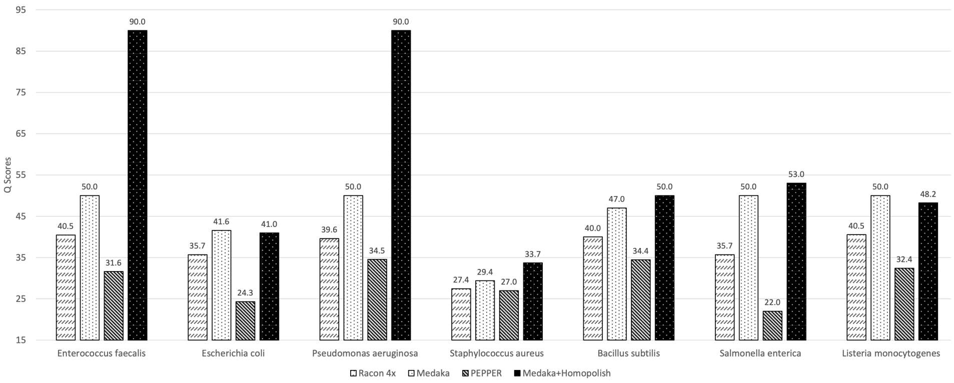
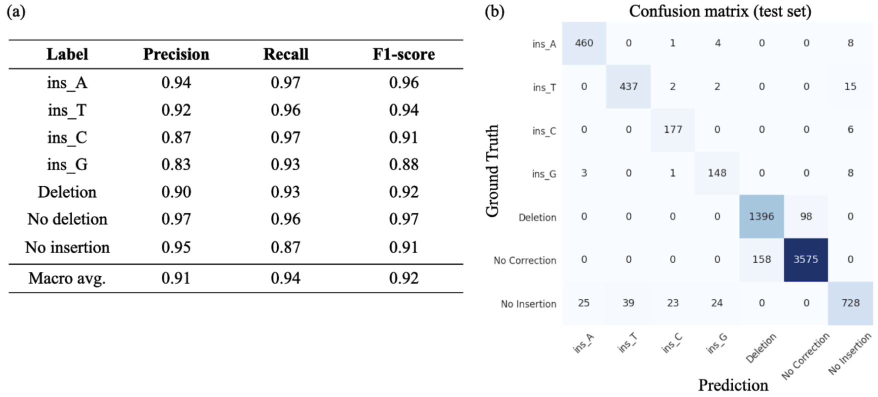


Fig. S19: Comparison of genome quality (Q score) polished by Racon, Medaka, PEPPER, and Homopolish on the R10.3 metagenomic dataset from Zymo Microbial Community Standard.


(a)
(b)
Confusion matrix (test set)
Ground Truth
Prediction

Label	Precision	Recall	F1-score
ins_A	0.94	0.97	0.96
ins_T	0.92	0.96	0.94
ins_C	0.87	0.97	0.91
ins_G	0.83	0.93	0.88
Deletion	0.90	0.93	0.92
No deletion	0.97	0.96	0.97
No insertion	0.95	0.87	0.91
Macro avg.	0.91	0.94	0.92

	ins_A	ins_T	ins_C	ins_G	Deletion	No Correction	No Insertion
ins_A	460	0	1	4	0	0	8
ins_T	0	437	2	2	0	0	15
ins_C	0	0	177	0	0	0	6
ins_G	3	0	1	148	0	0	8
Deletion	0	0	0	0	1396	98	0
No Correction	0	0	0	0	158	3575	0
No Insertion	25	39	23	24	0	0	728

Fig. S20: (a) Macro average of precision, recall, and F1-score of prediction of seven classes. (b) Confusion matrix of testing set during model training.