HiFi Based Metagenomic Assembly Strategy Provides Accuracy Near Isolated Genome Resolution in MAG Assembly

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Feilong Deng, Yanhua Han, Minghui Li, Yunjuan Peng, Jianmin Chai, Guan Yang, Ying Li, et al. 2025.
HiFi Based Metagenomic Assembly Strategy Provides Accuracy Near Isolated Genome Resolution in MAG Assembly. ***iMetaOmics*** 2: e70041. https://doi.org/10.1002/imo2.70041

Introduction

1. MAGs provide reference genomes for unculturable microorganisms

- With 715,230 bacterial species listed in the Genome Taxonomy Database (GTDB), many are represented by MAGs. (Parks et al., 2022)

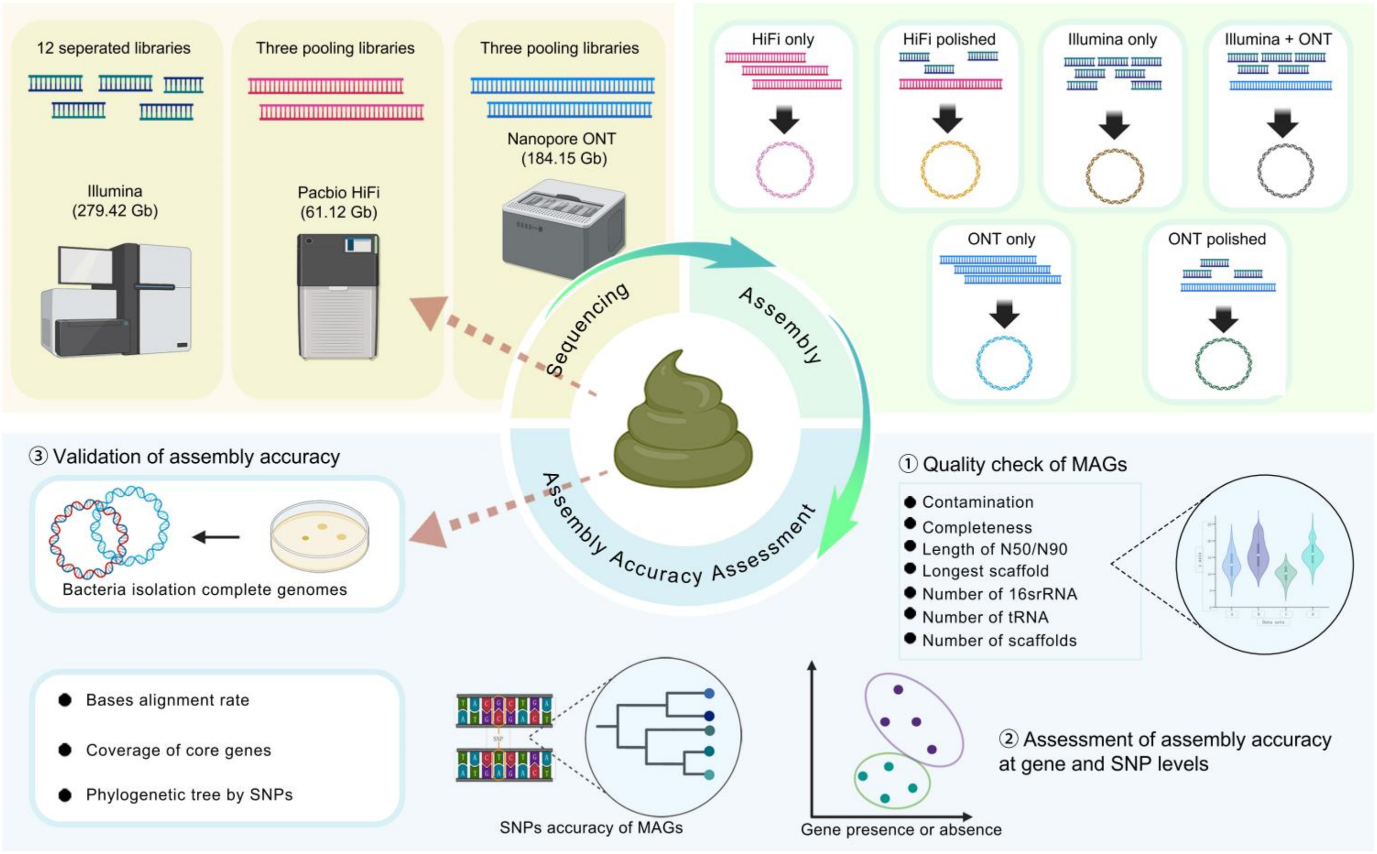
2. Challenges in MAGs Reconstruction

- **Assembly Quality**: Traditional short-read sequencing methods offer varied assembly quality, impacting the effectiveness of MAGs reconstruction.
- **Error Rates**: Third-generation sequencing technologies, while providing longer reads, come with higher error rates. (Karst et al., 2021; Watson and Warr, 2019)

3. Pacific HiFi may solve the existing problems in MAGs assembly

4. However, comprehensive comparison between different sequencing technologies for MAGs assemble is lacking.

Introduction



(1) Genome Evaluation Summary:

A comprehensive overview comparing the quality and completeness of assemblies.

(2) Comparison with NCBI Genomes:

Assembly results were benchmarked against genomes available in the NCBI database to assess accuracy and coverage.

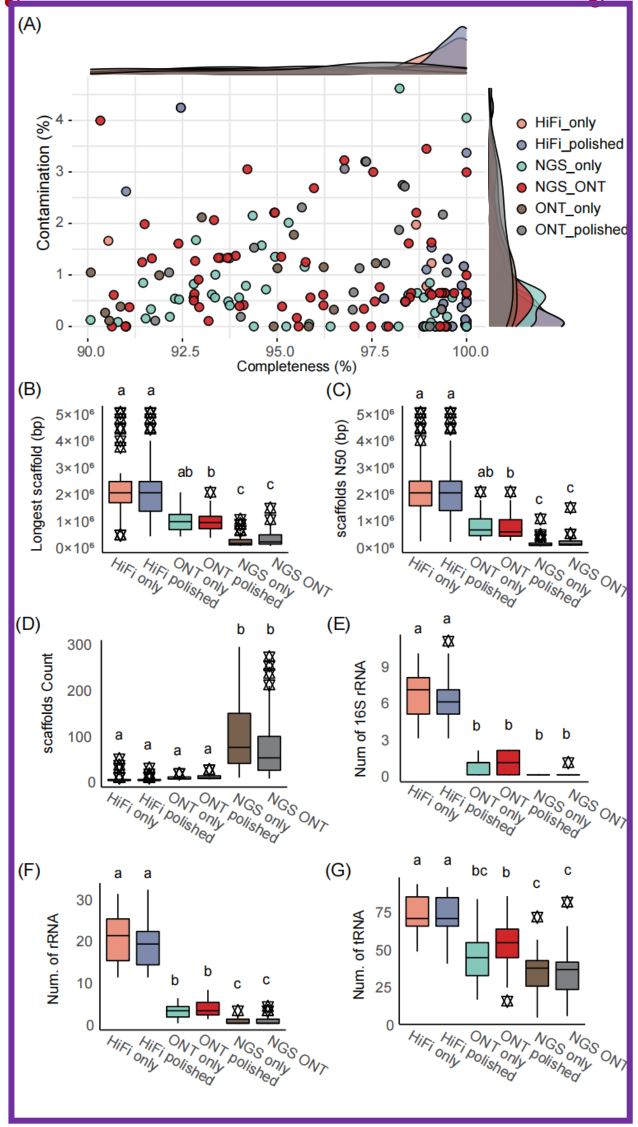
(3) Comparison with Isolate Genomes from the Same Sample:

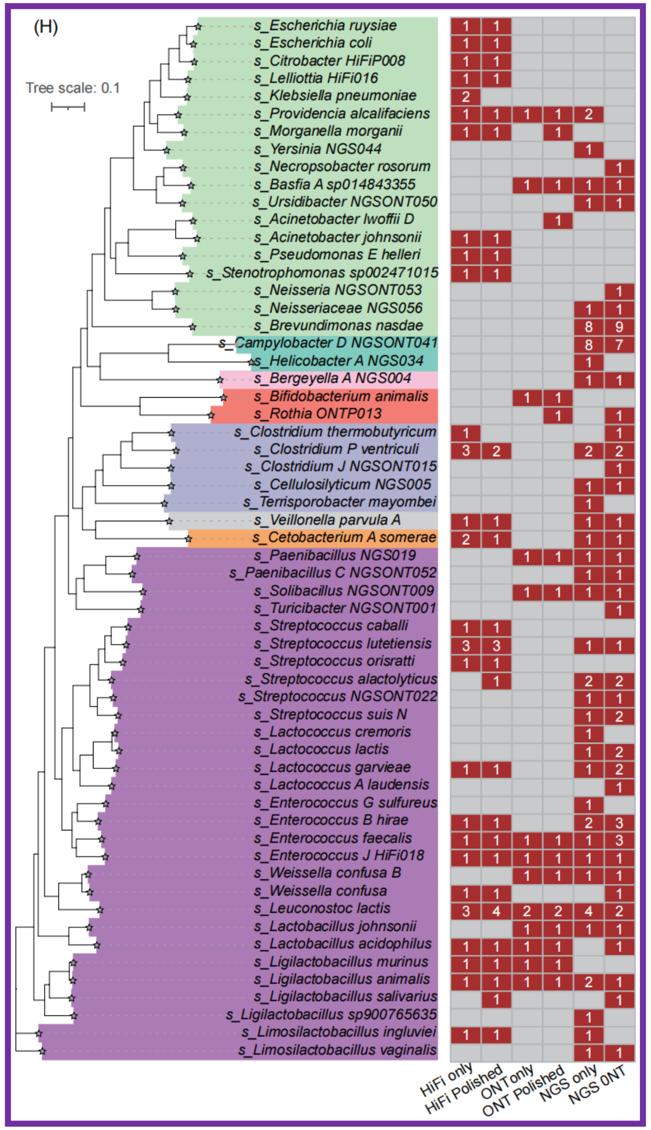
Direct comparison of the assembled genomes with isolates genomes obtained from the same samples.

Main Results

◆ Quality Comparison of MAGs by Different Assembly Methods

- ✓ A total of 59 species were assembled, among which four species were assembled by all six methods simultaneously.
- ✓ HiFi-related assembly offered higher MAGs completeness and contiguity, as well as lower genomic contamination.



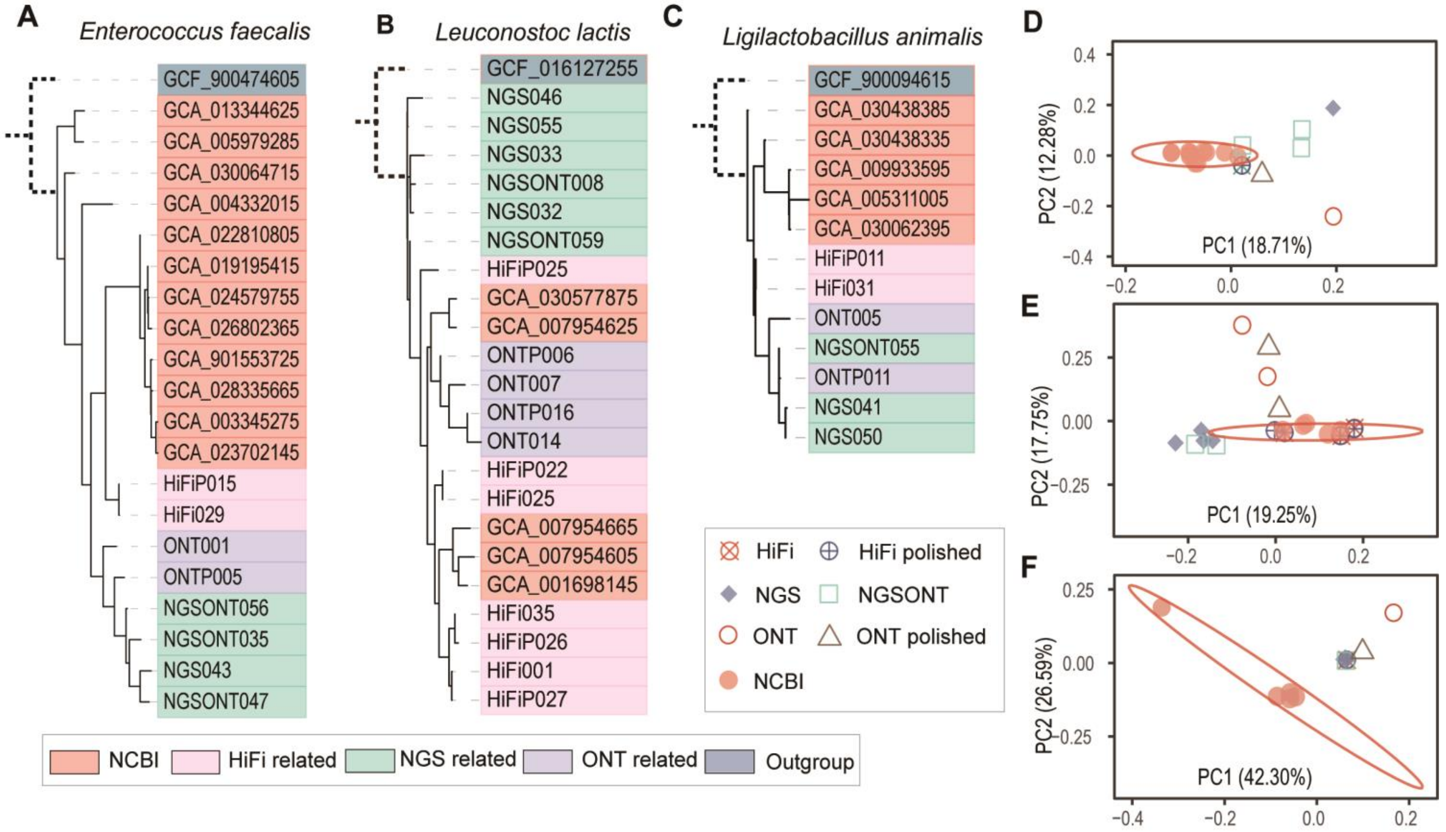


	HiFi only	HiFi Polished	ONTonly	ONT Polished	NGS only	NGS 0NT
s_Escherichia ruysiae	1	1				
s_Escherichia coli	1	1				
s_Citrobacter HiFiP008	1	1				
s_Lelliottia HiFi016	1	1				
s_Klebsiella pneumoniae	2					
s_Providencia alcalifaciens	1	1	1	1	2	
s_Morganella morganii	1	1		1		
s_Yersinia NGS044					1	
s_Necropsobacter rosorum						1
s_Basfia A sp014843355			1	1	1	1
s_Ursidibacter NGSONT050					1	1
s_Acinetobacter lwoffii D				1		
s_Acinetobacter johnsonii	1	1				
s_Pseudomonas E helleri	1	1				
s_Stenotrophomonas sp002471015	1	1				
s_Neisseria NGSONT053						1
s_Neisseriaceae NGS056					1	1
s_Brevundimonas nasdae					8	9
s_Campylobacter D NGSONT041					8	7
s_Helicobacter A NGS034					1	
s_Bergeyella A NGS004					1	1
s_Bifidobacterium animalis			1	1		
s_Rothia ONTP013				1		1
s_Clostridium thermobutyricum	1					1
s_Clostridium P ventriculi	3	2			2	2
s_Clostridium J NGSONT015						1
s_Cellulosilyticum NGS005					1	1
s_Terrisporobacter mayombei					1	
s_Veillonella parvula A	1	1			1	1
s_Cetobacterium A somerae	2	1			1	1
s_Paenibacillus NGS019			1	1	1	1
s_Paenibacillus C NGSONT052					1	1
s_Solibacillus NGSONT009			1	1	1	1
s_Turicibacter NGSONT001						1
s_Streptococcus caballi	1	1				
s_Streptococcus lutetiensis	3	3			1	1
s_Streptococcus orisratti	1	1				
s_Streptococcus alactolyticus		1			2	2
s_Streptococcus NGSONT022					1	1
s_Streptococcus suis N					1	2
s_Lactococcus cremoris					1	
s_Lactococcus lactis					1	2
s_Lactococcus garvieae	1	1			1	2
s_Lactococcus A laudensis						1
s_Enterococcus G sulfureus					1	
s_Enterococcus B hirae	1	1			2	3
s_Enterococcus faecalis	1	1	1	1	1	3
s_Enterococcus J HiFi018	1	1	1	1	1	1
s_Weissella confusa B			1	1	1	1
s_Weissella confusa	1	1				1
s_Leuconostoc lactis	3	4	2	2	4	2
s_Lactobacillus johnsonii			1	1	1	1
s_Lactobacillus acidophilus	1	1	1	1		1
s_Ligilactobacillus murinus	1	1	1	1		
s_Ligilactobacillus animalis	1	1	1	1	2	1
s_Ligilactobacillus salivarius		1				1
s_Ligilactobacillus sp900765635					1	
s_Limosilactobacillus ingluviei	1	1			1	
s_Limosilactobacillus vaginalis					1	1

Main Results

◆ Compared with NCBI genomes

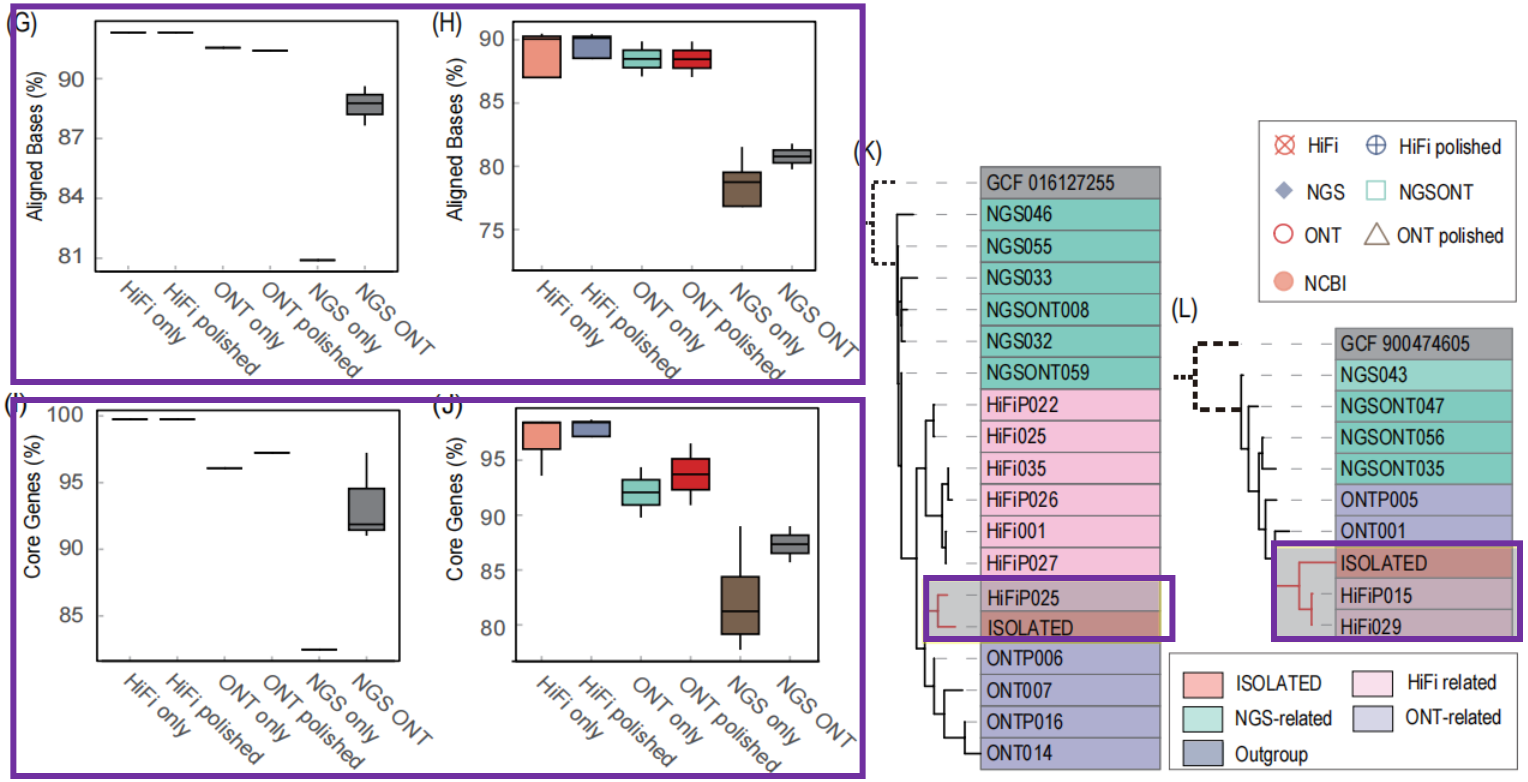
- ✓ MAGs from *E. faecalis*, *L. lactis* and *L. animals*, which were assembled by six assemble groups, were selected for further comparison.
- ✓ Compared with NCBI genomes, HiFi provides higher accuracy at both the single nucleotide and gene levels.



Main Results

◆ Compared with isolated genomes

- ✓ **Core Gene Coverage:** HiFi-based MAGs showed higher base and core gene coverage than those assembled by other methods.
- ✓ **Phylogenetic Analysis:** MAGs assembled with HiFi long reads were located within the same branch with isolates genomes, highlighting HiFi's superior assembly accuracy.



Conclusion

- **Key Findings:** HiFi assembly achieves precision comparable to bacterial genomes sequenced from pure cultures.

- **Implications:** HiFi metagenomics holds significant potential for future research requiring accurate microbial genomic data.

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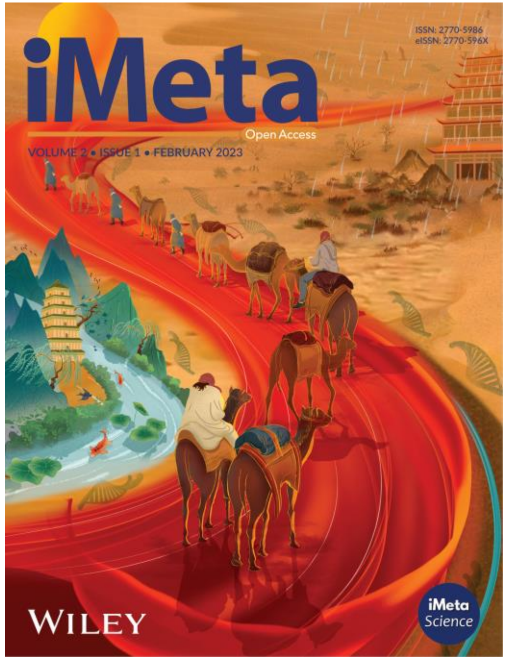

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