Efficient and easy-to-use capturing 3D metagenome interactions with GutHi-C

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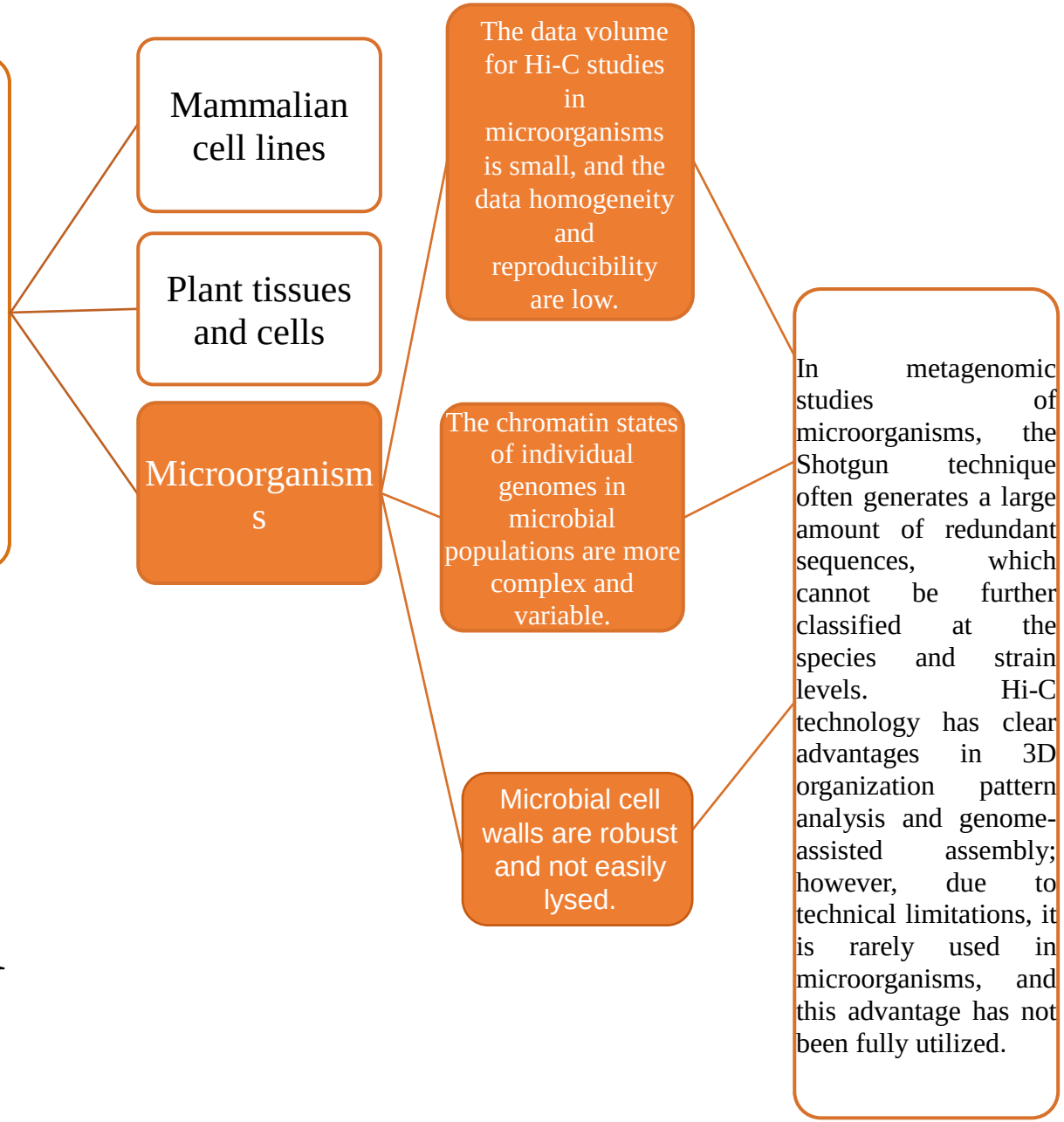
Background

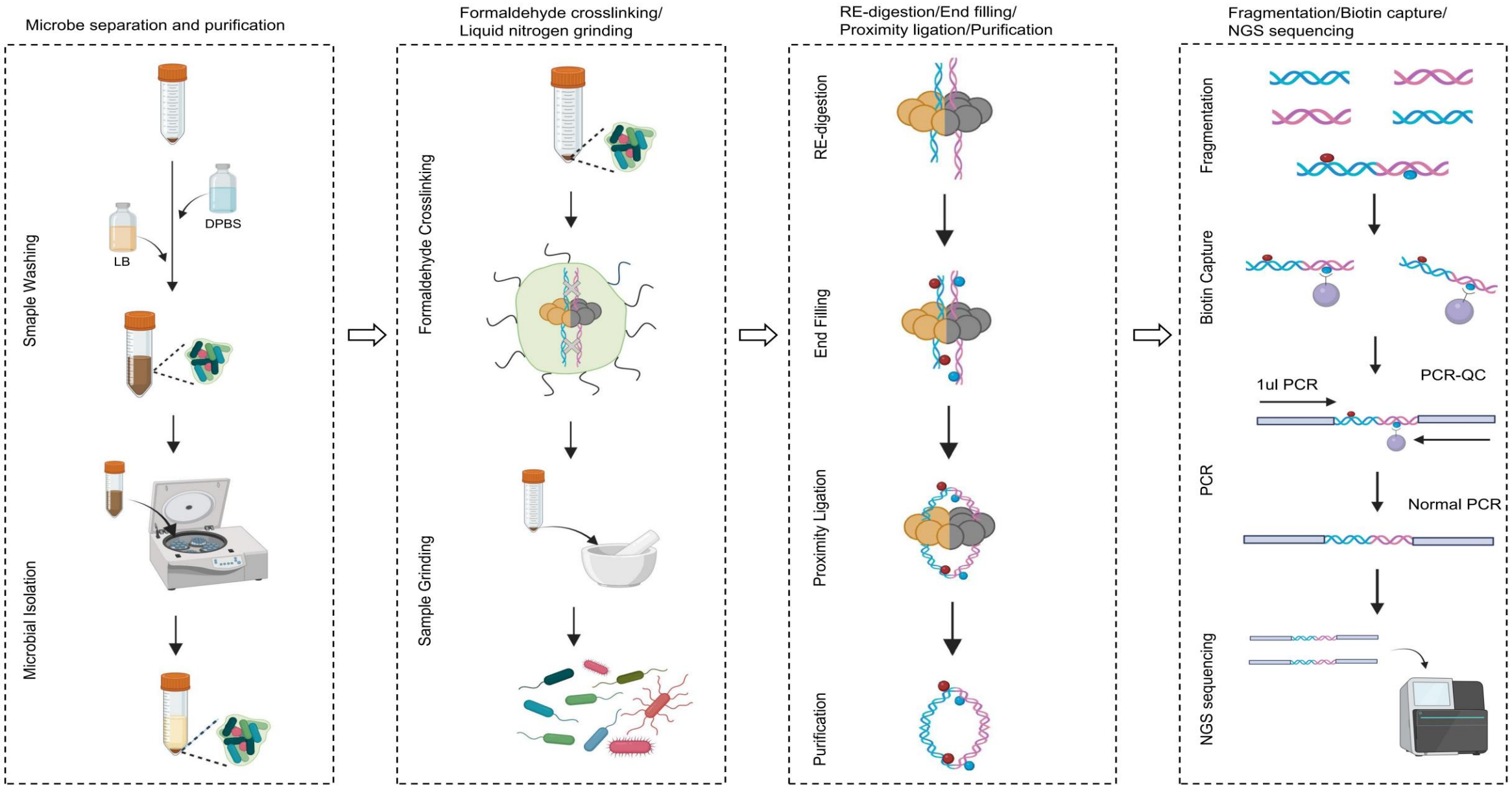
ned using animal cell lines that have the same cell type, chroma tin state



Highlights

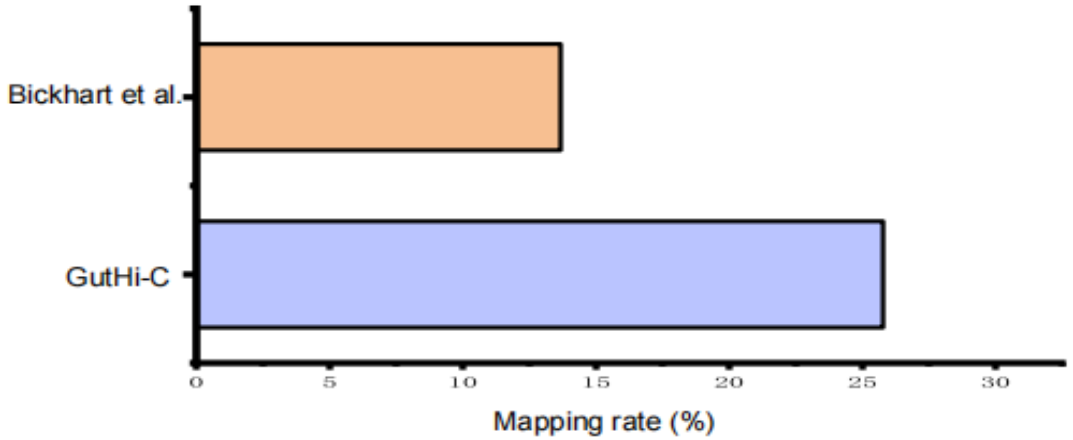
- This research provides a reliable, feasible, and practical metagenome GutHi-C constructing strategy, which is suitable for a wider range of microbial populations.
- It describes a detailed protocol of the GutHi-C technology. It is easy to handle and saves reagents. It also improves the efficiency of library construction and sequencing data quality.
- GutHi-C can be widely used for unraveling the 3D conformation of microorganisms, facilitating high-fidelity metagenomic assembly and other potential applications.

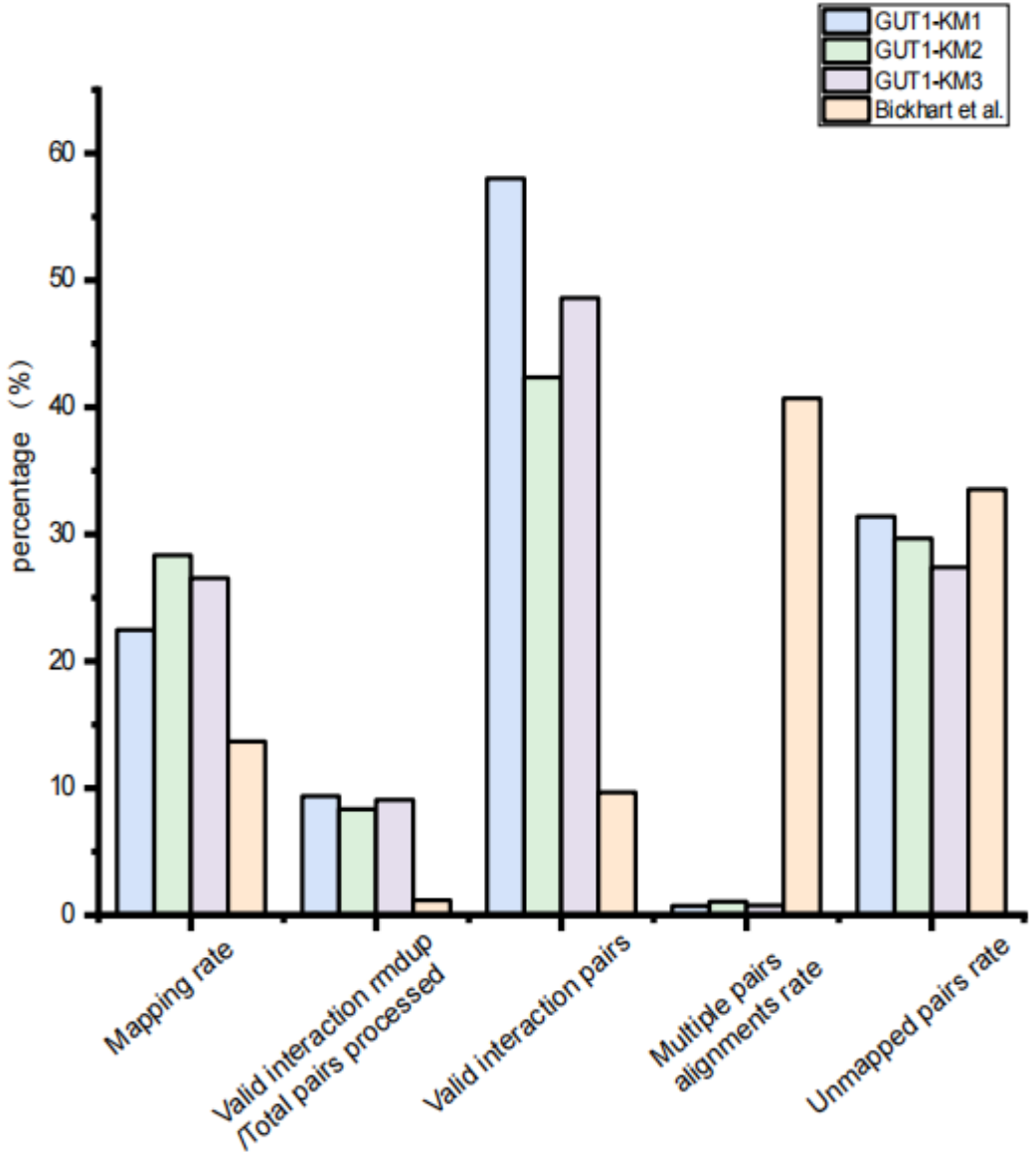
Overview of the procedure



Results

Data evaluation of GutHi-C technology



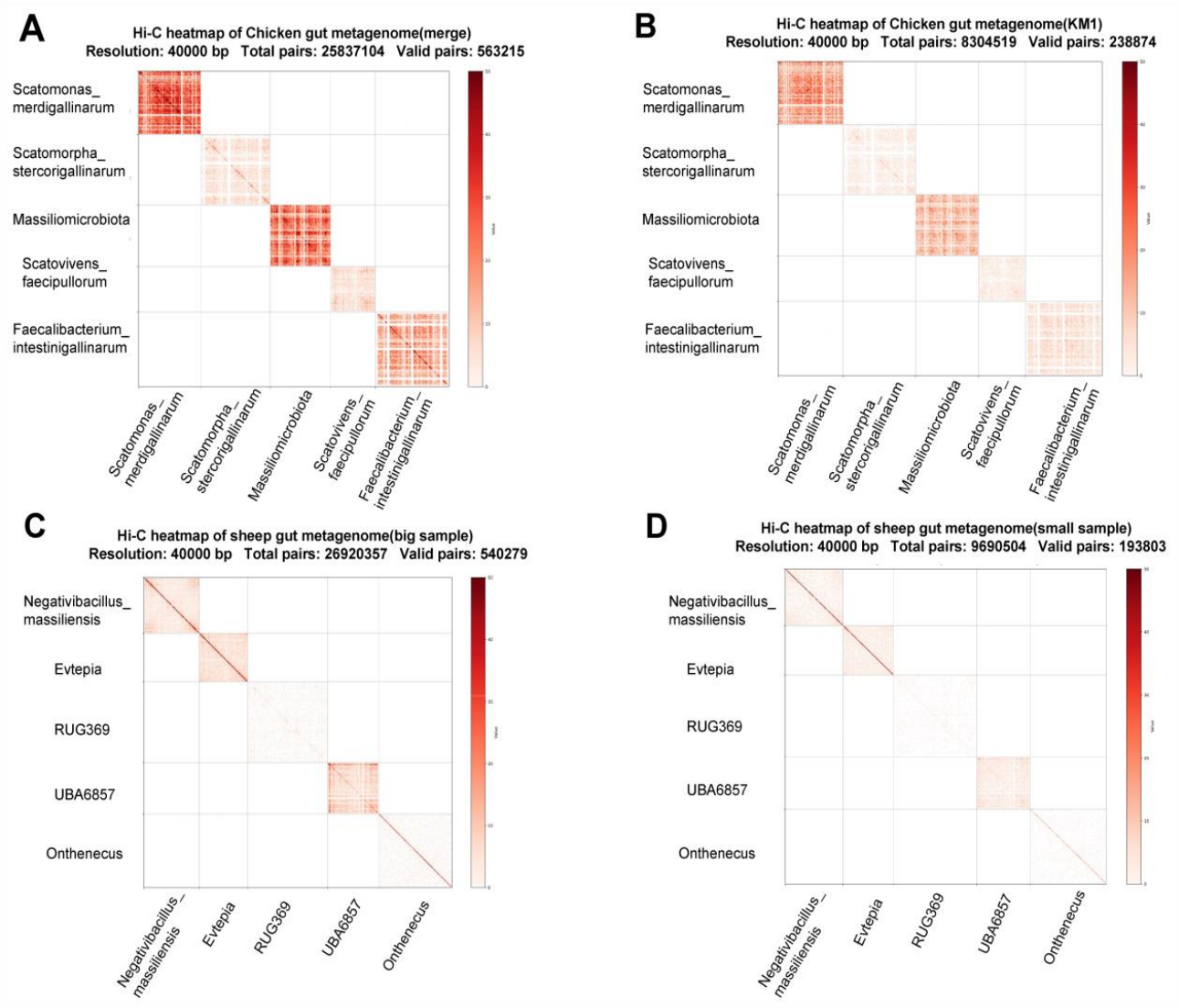


The library obtained in GutHi-C was sequenced (approximately 2 gigabases of raw data), and the data were evaluated by HiC-Pro after processing. The resulting assessments in Figure were compared with the ProxiMeta Hi-C (also known as Hi-C Meta) data in the previous work by Bickhart et al . Compared to ProxiMeta Hi-C data, GutHi-C indicates its favorable performance. Its data alignment rate (unique alignment rate), valid pair ratios and effective data yield rate are comparable to the data made by the pioneer method , which indicates that the GutHi-C library construction method in this research has more advantages and better quality.

Bickhart, D. M. *et al.* Generating lineage-resolved, complete metagenome-assembled genomes from complex microbial communities. *Nat Biotechnol* **40**, 711-719, doi:10.1038/s41587-021-01130-z (2022).

Results

Data evaluation of GutHi-C technology

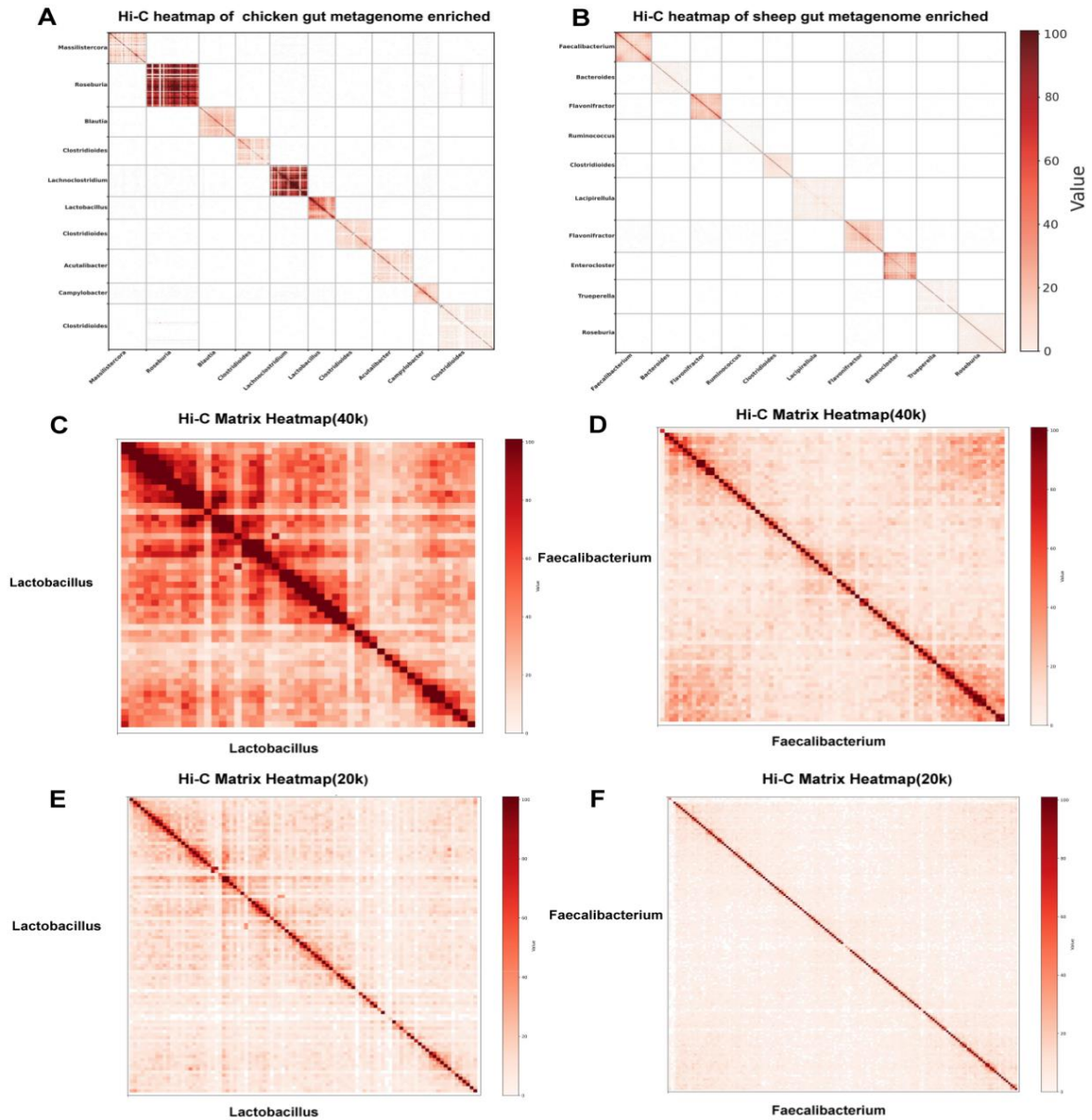


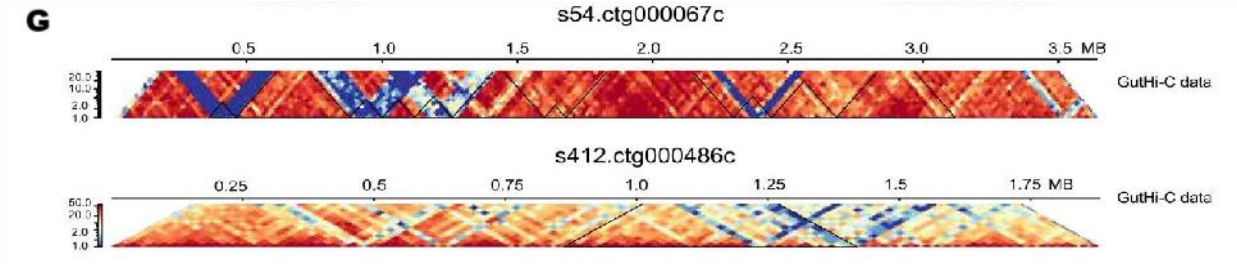
Employed interaction frequency heatmaps for comparative analysis of the dataset.

- The test data in this study, including GUT1-KM1, GUT1-KM2, and GUT1-KM3. On one hand, the control group was sampled to approximately 8 million total pairs (Figure D), and compared with GUT1-KM1 from this study (Figure B).
- On the other hand, the control group was sampled to approximately 25 million total pairs (Figure C), and compared with the combined data of GUT1-KM1, GUT1-KM2, and GUT1-KM3 from this study (Figure A)
- Comparative analysis reveals that, under equal data volume conditions, the methods used in GUT1-KM1 (Figure B) significantly outperformed the control group (Figure D). Furthermore, the interaction frequencies of the combined data of GUT1-KM1, GUT1-KM2, and GUT1-KM3 (Figure A) also surpassed those of the control group (Figure C).

Results

Application of GutHi-C to reveal the 3D conformation of microbial metagenomes





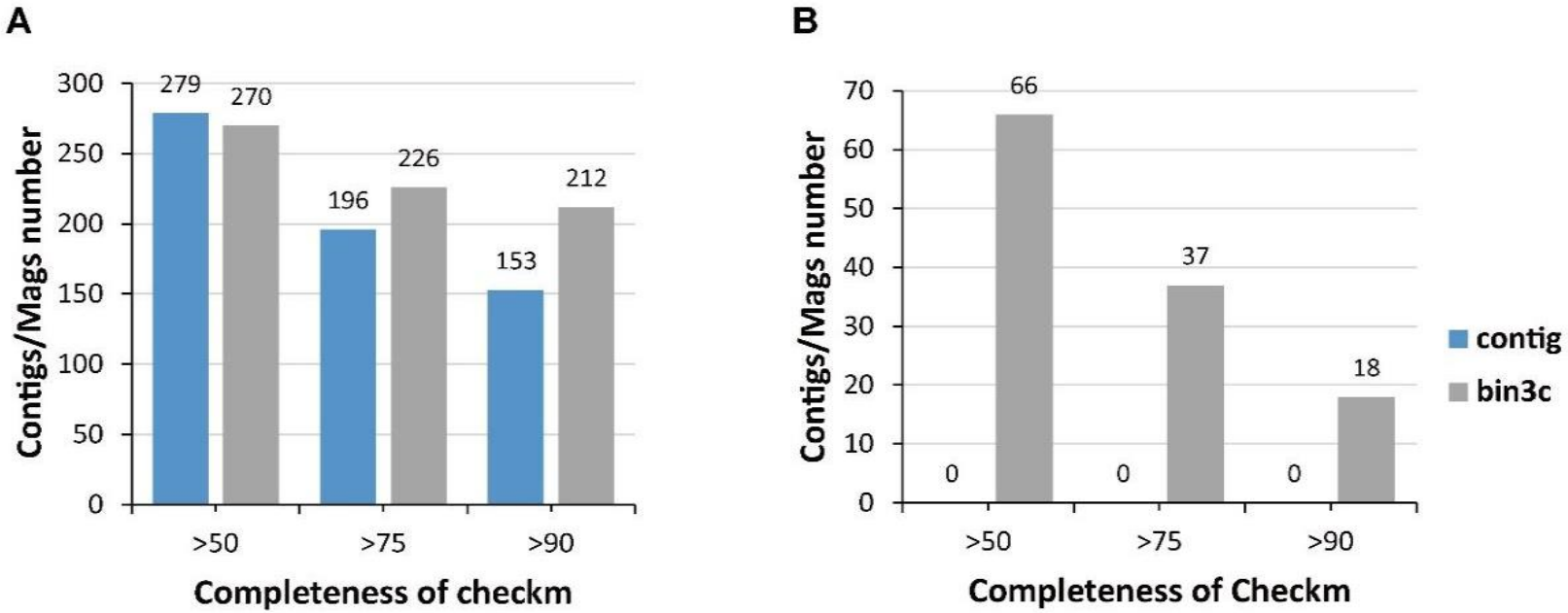
- We have re-collected the gut microbiota of the experimental chickens and reconstructed the GutHi-C libraries, which were submitted to large-scale sequencing (approximately 100~150 gigabases of raw data),We selected the top ten single bacteria from both our method and the control group ProxiMeta Hi-C for heatmap comparison. This comparison revealed that our method exhibited a more pronounced signal intensity

- Subsequently, we zoomed in on the bacteria with the strongest signal and showed higher-resolution plotting of the assembled and aligned genomes. It can be observed from both 40 kb bin and 20 kb bin resolutions that our method exhibits significantly stronger signal intensity (local interactions, also known as loops)

- As illustrated in Figure G, the region highlighted by the solid black triangle in the diagram represents an area of TAD-like strong interact frequency (chromosomal interaction domains, CID domains). Our results indicate there are regions within individual bacteria in the GutHi-C that represent strong interactive patterns.

Results

Application of GutHi-C to assist metagenome assembly combined with HiFi three-generation sequencing



Metagenomic Hi-C is mostly applied for metagenomic assembly/binning assistance, and currently, it has not been used for resolving the three-dimensional structures of microbes within metagenomes.

- The results, as shown in FigureA, demonstrate that owing to the good quality of GutHi-C data combining with high accuracy of HiFi, we obtained 212 high-quality metagenome-assembled genomes (MAGs) (Completeness > 90 and Contamination < 10) and 226 MAGs of medium to high quality (Completeness > 75 and Contamination < 10).This represents a 38.6% increase in high-quality genomes compared to the previous contig-level assemblies. However, the number of medium-quality MAGs (Completeness > 50 and Contamination < 10) has decreased. This suggests that using our Hi-C data allows for the classification of low-quality or medium-quality contigs, thereby enhancing the assembly quality of high-quality HiFi metagenomes.

- The difference is more apparent in FigureB, In the initial assembly of contigs using illumina TruSeq Shotgun sequencing, none achieved MAGs of medium quality,with the corresponding GutHi-C dataset, we obtained 66 medium-quality MAGs (Completeness > 50 and Contamination < 10), and 37 medium to high-quality and 18 high-quality MAGs.

Summary

- GutHi-C provides a metagenomic Hi-C library preparation method suitable for microbial communities. Based on the common characteristics of microbial communities, this technology can be applied to microbial populations from various environments, such as those found in the guts and feces of livestock, humans, and other animals, soil microbial populations, and microbial mixtures in culture. It offers an efficient approach for constructing large microbial genome libraries and high-throughput sequencing, presenting a promising application prospect for metagenomic library construction and high-throughput sequencing.
- GutHi-C technology include microbial cell lysis performed through a combination of liquid nitrogen grinding and lysozyme treatment. This approach maximizes the dissociation of microbial cell walls, facilitating optimal penetration of endonucleases into the cell nucleus. While introducing the in situ Hi-C system, the usage of biotin and magnetic beads was reduced. Moreover, PCR quality control (PCR-QC) test is carried out before DNA formal amplification. It can obtain the optimal amplification conditions, improve the preparation ratio of the GutHi-C library, and avoid reagent waste.
- In terms of assembly applications, comparisons with ProxiMeta Hi-C demonstrate that using the Hi-C data generated by this method can improve the classification of low-quality or medium-quality contigs，it can be used to assist in the assembly of high-quality metagenomes of microorganisms

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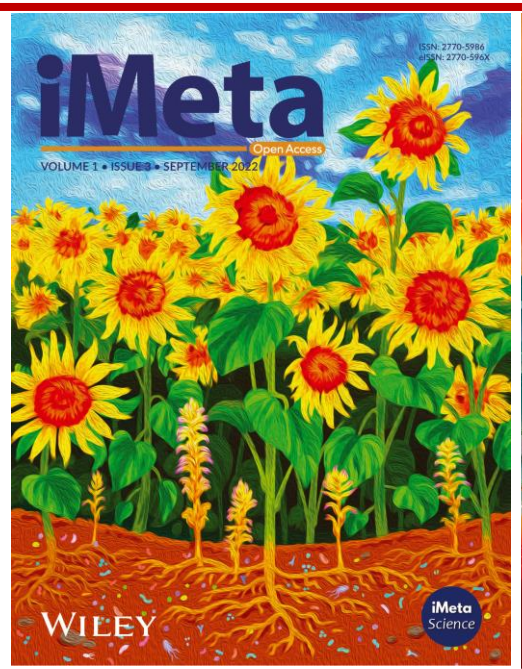

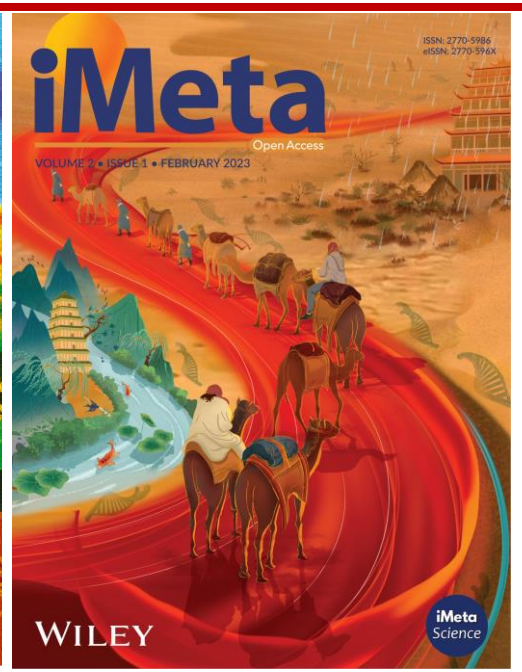

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