

## **Comparing and evaluating metagenomic HiFi read assemblers**

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As Next Generation Sequencing technologies develop, shotgun sequencing approaches are currently being used as the most representative method for microbiome research. Also, recent advances in bacterial sequence binning methods enabled studying of unculturable microbial genomes through metagenome assembled genomes (MAGs). Especially in the gut, given that < 20% of the known gut commensal species have been cultured, research on the unculturable microbial genome using MAGs is of great importance. Currently, most metagenomic sequencing data from shotgun sequencing are available as short-read sequences. Despite high accuracy, short read sequencing technologies have an intrinsic drawback in de novo genome assembly due to the limited read length. Alternatively, we may use long-read sequencing to overcome the length limitation, but it suffers from poor accuracy. Therefore, hybrid assembly methods, which combine strength of short reads in accuracy and long reads in assembly, have been proposed as the best practice for reconstruction of microbial genomes from the complex community.

Recently developed PacBio HiFi sequencing was reported to complement the shortcomings of previous long-read sequencing by achieving high accuracy through circular consensus sequencing (CCS). Therefore, we can use HiFi sequencing to assemble much more conveniently than hybrid assembly. Fortunately, several software tools are available for metagenomic assembly of HiFi reads. However, it can be a bit difficult to figure out which tool performs best biologically. To provide a guide for the metagenomic assembler of HiFi reads for microbiome researchers, we benchmarked three HiFi read assemblers (HiCanu, metaFlye, hifiasm-meta) in terms of computing power, contig level, and bin level using four public HiFi sequenced fecal samples provided on the PacBio website.