

## **Novel genetic features in human gut microbiomes uncovered by long-read metagenomics**

Masahira HATTORI, Wataru SUDA

Laboratory for Microbiome Sciences, RIKEN Center for Integrative Medical Sciences

Hundreds of trillions of microorganisms inhabit the human gut and form the gut microbiome, which are profoundly associated with host physiological states including disease. Thus, it is obviously important to precisely evaluate the whole pictures of microbiome structure in the human microbiome research. To this end, our laboratory is working on various technological developments in NGS (next-generation sequencing)-based metagenomic analysis of human microbiomes including gut, oral, and skin.

In most of previous studies, short reads of ~300-bp produced by Illumina sequencers were used in metagenomic analysis of the human gut microbiome. However, short-read sequencing has several intrinsic limitations for completeness of analysis largely due to existence of high-similar repetitive sequences such as ribosomal RNA genes in microbial genomes, sometimes resulting in ambiguous or insufficient outcomes.

Here, we present metagenomic sequencing of human gut microbiomes with a long-read PacBio sequencer, generally producing long reads of ~10-kb in genome sequencing. Our data showed that reads of ~9-kb on the average were successfully obtained from 12 human fecal DNA samples, and the microbial abundance quantification was equivalent to that by short-read sequencing. Assembly of PacBio reads, a total of ~10-Gb per sample, followed by binning of contigs reconstructed ~100 high-quality chromosomal bins up to 6.8-Mb in size including seven complete circular chromosomal contigs without binning. Additionally, we also identified ~90 small circular contigs up to 667-kb in size, all of which were assigned to extra-chromosomal plasmids and phages, more than half of which were novel but prevalent in human gut microbiomes. Thus, long-read metagenomics is a powerful approach to uncover complex genetic features of the human gut microbiome.