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Critical advances for amplicon sequencing in metagenomic research

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Profiling microbial communities through sequencing rRNA amplicons is a cornerstone of metagenomics, and a potential important component of monitoring biological contamination in a fermentation environment. We describe cost-saving and accuracy-improving innovations for amplicon sequencing on the Illumina MiSeq, most of which are applicable to other platforms. First, we use PCR clamps to eliminate contaminating sequences from a host organism without biasing the microbial community. Second, we incorporate frame shifts into our primers to increase diversity and base-calling accuracy at each sequencing cycle, and to eliminate the current wasteful practice of sequencing up to 50% PhiX genomic DNA. Finally, we break the amplicon PCR into two rounds. Round-1 uses only two cycles to tag template molecules. Round-2 amplifies the tagged templates and adds universal sample indexes. The template tags allow error-correction and reduction of PCR bias through consensus building. Furthermore, because the round-2 index primers are not gene-specific, the same set of indexes can be used to study different amplicons. As an additional capability, a small set of sample barcodes on the round-1 template tagging primers can be paired with round-2 indexes to multiplicatively increase the indexing depth. Together these techniques improve both the efficiency and accuracy of rRNA amplicon sequencing.

Biography

Piotr Mieczkowski has completed his Ph.D. at the Institute of Biochemistry and Biophysics Polish Academy of Sciences and postdoctoral studies at the University of North Carolina at Chapel Hill and Duke University. He is the Director of the High Throughput Sequencing Facility at UNC. He has published more than 48 papers in reputed journals. His work is focused around new applications for next generation sequencing technology, genome stability and mutagenesis.

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