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Researchers Use 454 Sequencing System for Rapid Sequencing of Avian Influenzas

A team of researchers at the Institute of Diagnostic Virology, Friedrich-Loeffler-Institute Insel Riems, in Germany has developed a simple and rapid method for preparing Avian Influenza samples of infected individuals for sequencing with the Genome Sequencer FLX system.

The highly pathogenic avian influenza A virus (HPAIV) of subtype H5N1 has caused global concern as a potential pandemic threat, killing millions of poultry and fatally crossing over to humans in a number of documented cases. The risk of sequence mutations which cause an increase in pathogenicity, meaning an escalation in the health threat of the virus, demands fast and reliable methods for in-depth full-length sequence analysis to prevent spread of the disease. Höper et al. (1) designed a simple and sensitive method for the preparation of sequencing libraries from HPAIV H5N1 RNA samples for sequencing with the Genome Sequencer FLX instrument. The presented method seamlessly integrates high-throughput 454 sequencing into analysis without necessitating additional equipment or molecular biological techniques besides standard PCR and the Genome Sequencer FLX sample preparation and sequencing pipeline.

According to the authors, their approach is the first published method enabling sequencing of complete HPAIV H5N1 genomes directly from individual human samples using the Genome Sequencer FLX. Massively parallel sequencing of shotgun libraries with the GS FLX System with standard series reagents generated up to 500 Mb of raw sequence data in a single instrument run. The tremendous depth of coverage provided by the sequencing data enabled representation of every nucleotide in multiple independent reads.

“This method is simply not comparable with conventional sequencing and by far surpasses the single reads of ‘diagnostic’-relevant sites of the hemagglutinin gene of avian influenza as generated in former studies,” explains Dirk Höper. “Our method allows for sequencing of complete HPAIV H5N1 genomes from routine samples, neither requiring previous virus propagation in eggs or cell culture nor cloning and amplification of cDNA in vectors. Our approach permits sequencing of up to 8 complete viral genomes within only 3 days at an unprecedented depth and consequently,

reliability of the sequence. The procedure has the potential to seamlessly integrate into the normal diagnostic routine.” The described procedure can be easily adapted to different HPAIV subtypes, allowing sequencing of full length genomes immediately after identification of the subtype by routine diagnostics.

“The high accuracy and quick run-time make 454 Sequencing ideal for medical applications. This study demonstrates the tremendous potential our technology has for future diagnostic applications in the field of virology,” says Chris McLeod, President and CEO of 454 Life Sciences.

454 Life Sciences, a center of excellence of Roche Applied Science, develops and commercializes the innovative 454 Sequencing system for ultra-high-throughput DNA sequencing. Specific applications include de novo sequencing and re-sequencing of genomes, metagenomics, RNA analysis, and targeted sequencing of DNA regions of interest. The hallmarks of the 454 Sequencing system are its simple, unbiased sample preparation and long, highly accurate sequence reads, including paired-end reads. The technology of the 454 Sequencing system has enabled hundreds of peer-reviewed studies in diverse research fields, such as cancer and infectious disease research, drug discovery, marine biology, anthropology, paleontology and many more.

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(1) Höper D, Hoffmann B, Beer M. Simple, sensitive, and swift sequencing of complete avian influenza H5N1 genomes, J. Clin. Microbiol. 2009 47: 674-679.

The 454 GS FLX is sold for life science research use only. The 454 Genome Sequencer FLX is intended only for use in basic or applied laboratory research, and is not intended for use in human diagnostics. Roche Diagnostics does not condone any use of the 454 Genome Sequencer FLX other than basic or applied laboratory research, and Roche Diagnostics will not support any other use.

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