PREPARATION OF HALOPLEX CARDIOGENETIC PANEL FOR TARGETED SEQUENCING OF HEART RHYTHM DISORDERS

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Introduction. Next generation sequencing allows laboratories to detect genetic changes in target genes associated with specific disorders effectively and quickly. Today, Agilent Technologies offers two panels with pre-selected content - HaloPlex Cardiomyopathy and HaloPlex Arrhythmia, that consist of 34 and 21 genes, respectively. However, these cardiogenetic panels do not consider all genes associated with rhythm disorders.

Aim of the project: To create the new HaloPlex cardiogenetic panel of sequencing that consists of 96 genes associated with arrhythmias using HaloPlex (Agilent Technologies) technology for differential diagnostics of heart rhythm disorders.

Materials and methods. SureDesign Online Design software (Agilent Technologies) was used to create HaloPlex cardiogenetic panel for targeted sequencing of 96 candidate genes. DNA-libraries for 90 patients with cardiac arrhythmias were prepared using HaloPlex Custom Panel Tier 1 kit (Agilent Technologies) according to the manufacturer's protocol 'HaloPlexTarget Enrichment System for Illumina Sequencing', v.D3. December 2012. The protocol is optimized for digestion of 225 ng of genomic DNA. Enrichment Control DNA was used as a control. Quality and quantity of 90 samples were estimated using Qubit 2.0 and 2% Agarose gel. Human Genome version 19, GRCh 37, February 2009 for Illumina platform was applied for preparation of final design.

Results. New HaloPlex cardiogenetic panel for targeted sequencing of 96 genes associated with rhythm disorders was developed using SureDesign Online Design software (Agilent Technologies). Developed panel was downloaded and all targets were estimated using UCSC Genome Browser. Size of the target region was 463.767 kbp, length of reads - 150 bp. 19958 amplicons were created by the software to cover all target regions. 99.46% of all target regions were covered successfully. To prepare DNA libraries of candidate genes firstly DNA samples were fragmented by restriction enzymes, then probe library was hybridized to both ends of target fragments to create circular DNA molecules. 90 samples were indexed with different indexes. Circular DNA molecules were joined together in the ligation reaction. Then, target fragments were PCR-amplified and sent for sequencing.

Conclusion. 90 DNA-libraries were prepared using created HaloPlex cardiogenetic panel. All samples were sequenced on Illumina HiSeq2000 platform. Bioinformatic analysis of obtained sequencing data is being conducted.