ABSTRACT

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The endeavor to sequence the whole human genome lead not only to the knowledge acquisition regarding the human genetic information but as well to the development of new sequencing methods and technologies. In order to keep up with progress in genetic field in many clinical and research laboratories the new massive parallel sequencing equipment is being utilized. On the market are currently established four leading platforms – Illumina, Solid, Ion Torrent and 454 Life Technologies. The process of sequencing analysis can be summarized into three main steps – the sequencing library preparation, sequencing itself, variant calling and data analysis. Each part of the sequencing analysis exhibits certain specifics, we need to count with and as well its pitfalls, we need to avoid or to minimalize their impact on the analysis final result. Recently new methods termed sequencing of the 3rd generation are being developed, enabling sequence of a single DNA molecule to be determined without previous amplification. NGS technologies provide those days laboratories with ability to choose their own preferences in sequencing, where the laboratory can choose whether it needs data obtained by whole genome sequencing, exome sequencing or whether to test certain group of genes in so called panel.

In the practical part of this study is described the establishment of next generation sequencing in the diagnostic laboratory and optimalization of required processes. The trial group of 191 patients with diagnosis of hereditary cardiomyopathy was selected in collaboration with cardiology clinic of IKEM and University Hospital Motol in order to detect germinal mutations. In 65 patients the TruSight Cardiomyopathy panel was utilized, and causal mutation was uncovered in 48 patients. In remaining 126 samples comprising 42 families the clinical exome was sequenced using TruSight One kit and revealing causal mutation in 27 more families. The usage of NGS methods was tested as well in group of 8 patients from 3 families with serious genetic condition, where expanded exome probes covering 37 Mb were employed.