Abstract

This diploma thesis provides an overview of gene conversion, its role in the pathogenesis of human diseases and the use of methods based on next-generation sequencing (NGS) for detection rare variants of DNA sequence. Labeling of target DNA molecules by random nucleotides in primer and NGS were used for detection point mutations arising *de novo* in the β-glucocerebrosidase gene by gene conversion between it and its pseudogene in meiotic and mitotic cells of control subjects. Primers specific for the active gene were used to selectively amplify the ninth and tenth exon of the gene where "recombinant" variants occur most frequently.

Sequences generated from 20 genomic DNA samples on Illumina MiSeq platform were quality filtered, sorted by unique labels and consensus sequences were created from alignments of sequences carrying the same DNA tag. The number of potential point mutations in the samples ranged between 12 and 48. The mutations were manually re-evaluated from the alignments. The number of alignments with unique labeling was in the range of 7-15 thousand per sample. Only three samples carried possible recombinant mutations, suggesting a lower frequency of conversion in the region than reported by other techniques. Analysis of unique sequences in primer indicated possible ways to improve the sensitivity of the method.

Another goal was to test methods of preparing the template for NGS by enriching the converted sequences. Methods based on padlock probes, mutational-specific PCR and loop-mediated isothermal amplification yield sufficient amounts of good quality template for NGS.

Key words: gene conversion, β-glucocerebrosidase gene, β-glucocerebrosidase pseudogene, next-generation sequencing, point mutation