Multiple publications showed that many genes in human and other genomes share the same fragment of DNA sequence and form so called pairs of overlapping genes. This phenomenon is believed to have an important regulatory role. However, results of so far performed studies are not consistent and in majority are not supported by experimental validation. Therefore, the main goal of the intended study is to investigate expression regulation mechanisms of proteincoding genes located on opposite DNA strands in head-to-head orientation. In the course of our studies we hope to answer the key question if such genes organization evolved as regulator of genes expression or is just a byproduct of other processes. To address this we will perform extensive analysis of overlapping genes expression. Data from state of the art technologies will be used for bioinformatics analyses and ground breaking experimental methods will be utilized to validate results. Performed experiments will contribute to the development of new analytical approaches and obtained results will provide new insights to our understanding of the protein-coding genes overlap phenomenon. It will also significantly contribute to our knowledge about gene expression regulation. Results of proposed studies will not only have cognitive value but also will be of big importance to biomedical sciences, particularly for studies on genes directly or indirectly involved in human diseases since it was demonstrated that many overlapping genes do contribute to various diseases. A substantial part of proposed studies is focused on alternative promoters. The functional significance of alternative promoters is largely unexplored but it was shown that aberrant use of alternative promoters might give rise to cancer. Therefore, the study would also have impact on our understanding of a role of promoter shift in induction of diseases.