

## **Abstract**

This work is focused on reverse genetics of anaerobic protists, mainly *T. vaginalis*, *G. intestinalis* and *E. histolytica* and deals with techniques and experimental procedures of genome manipulation in these parasites.

Both DNA and RNA can be manipulated and the gene function can be disclosed using methods of reverse genetics. The knowledge gained is useful in many ways. For example, using these techniques crucial aspects of biology of parasitic protist are studied, providing basis for potential development of new drugs. Utilization of such methods also helps to understand the cellular and metabolic pathways and mechanisms, that could be very diverse or reduced in protists.

The methods of reverse genetics that result in permanent and inheritable changes in DNA are, for instance, homologous recombination or DNA integration. There is also a transcriptional gene silencing (TGS) technique to stop gene expression even though the coding DNA remains unchanged. TGS could be realized by several mechanisms, for example by RNA interference. RNA interference pathway, commonly known as posttranscriptional gene silencing mechanism, causes the breakage of mRNA or stops its translation. Other techniques of gene silencing involve, e.g., the expression of antisense RNA, oligonucleotides and ribozymes.