

Retroviral vectors are used as mighty tools for an introduction of recombinant genes into the recipient genome in gene therapy trials. In the vector design, great emphasis is put on safety and efficiency. In spite of a great progress in retroviral vector design with the purpose to stabilize its expression, e.g. introduction of protective elements into the viral regulatory sequences, the current approaches are still not sufficiently effective and the majority of proviruses is transcriptionally silenced. The understanding of the silencing mechanism is of special importance to the optimization of the vector design and handling. In this master thesis, I have designed and constructed an expression system for study of the mechanism involved in the silencing of retroviruses integrated inside gene bodies. This artificial system will be utilized for testing of hypothesis that retroviruses integrated into gene bodies are silenced by DNMT-dependent mechanism and this process is triggered by transcriptional read-through of the provirus from nearby host promoter. I have obtained preliminary results suggesting the validity of the suggested hypothesis; however the verification of general validity of this hypothesis for various retroviruses and elements will be a matter of further studies in our laboratory.