Abstract of Aliaksei Papkov's doctoral dissertation entitled: "Changing the substrate specificity of the thermostable restriction endonuclease TthHB27I by genetic engineering of the catalytic motif and cofactor-binding motif".

My project aim is to change DNA substrate specificity of the bifunctional restriction endonuclease-methyltransferase TthHB27I, which is atypical, subtype IIS/IIG/IIC REase belonging to the Thermus sp. family. Our team's previous findings demonstrate that several REases from Thermus sp. family exhibit the unique “affinity star activity” - the relaxed sequence recognition. Secondary (“star”) activity is a phenomenon demonstrated by several type II REases subjected to reaction conditions such as high pH, low ionic strength, the presence of other organic solvents and metal ions other than magnesium, excess of enzyme units. Moreover, the cleavage activity and cognate site recognition could be affected by cofactor SAM (S-adenosylmethionine) and its analogue SIN (sinefungin). Sequence analysis of Thermus sp. enzymes defined crucial, most conserved motifs implicated in maintenance of MTase and REase activity: common nuclease active site – PD-(D/E)XK, SAM binding site and methylation catalytic motif NPPW/Y. Accordingly, to the sequence similarity in these motifs members of Thermus sp. are divided into two groups: TspGWI subfamily (TspGWI and TaqII) and TspDTI subfamily (TspDTI, Tth111II/TthHB27I, TsoI). It is shown that cleavage activity and specificity of TspGWI and TaqII were changed in presence of SIN, in case of SAM specificity relaxation was not observed, although restriction activity was dramatically increased by both SIN and SAM. The hypothesis, which will be verified in my project, is whether substrate specificity relaxation of the TthHB27I restriction endonuclease will be affected by sequence changing in the crucial motifs. The enzyme utilised in my thesis is a product of synthetic gene, coding for the recombinant restriction endonuclease TthHB27I, possessing the identical to native amino acid sequenced version. The new versions of mentioned TthHB27I may be more responsive to the presence of SIN/SAM. If the specificity relaxation occurs, it will provide to targeted “affinity star” activity.

Mutagenesis will be performed by PCR site-directed mutagenesis. Primers with desired mutations will be designed using bioinformatics tools. Native TthHB27I sequence in SAM binding site DPACGSG will be converted into DPAVGTG – presented in TspGWI and methylation catalytic motif NPPW will be changed into NPPY - motif presented in both TspGWI and TaqII. Three versions of mutants will be obtained using the site-directed mutagenesis: two mutants with single mutation: NPPY mutant or DPAVGTG mutant, and one

with both changed motifs DPAVGTG and NPPY. Mutations will be verified using restriction enzymes and next sequencing. New forms of enzymes after the expressing in *E. coli* strain will be isolated, purified by ion-exchange chromatography and biochemically characterized.

The main application of frequently cleaving restriction enzymes is a genomic libraries preparation. Genomic library is necessary for the following sequencing of whole genome. Over the last decade the use of sequencing rapidly grew and its cost decreased. It is associated with the advancement in next-generation sequencing technologies (NGS), which are replacing traditional Sanger method variants. Number of NGS-related articles available in Medline grows exponentially. It confirms the fact that NGS technologies have demonstrated their enormous potential for scientists in field of medicine, biology and other life sciences. The initial step for all NGS techniques is a high molecular weight DNA fragmentation. The size of obtained DNA fragments is a key parameter for the construction of genomic libraries for NGS. There are three basic DNA fragmentation approaches: physical, enzymatic and chemical. However, enzymatic method seems to be the method of choice, because of its simplicity and reproducibility.

Apparently, the demand for new frequently cleaving restriction enzymes will be constantly growing. Therefore, the development of new enzyme systems becomes a today current interest. I think, my project has a high probability of success in “molecular scissors” development. Even in case of hypothesis discard, valuable data for further studies will be obtained.