

Summary of Edyta Czajkowska's PhD thesis: Identification, cloning and expression of the new *taqIIIRM* gene from thermophilic bacteria *Thermus aquaticus* on the basis of bioinformatic analysis of the genome sequence obtained by new generation sequencing methods.

Thermophilic bacteria *Thermus aquaticus* (*T. aquaticus*) belong to the genus *Thermus*. These bacteria inhabit hot springs, the temperature of which oscillates between 55-95°C. In 1984 Barker *et al.* isolated and characterized the enzyme RM.TaqII (Barker *et al.*, 1984). They have shown that this enzyme specifically recognizes two DNA sequences 5'-GACCGA-3' and 5'-CACCCA-3', and has two enzymatic activities located in one polypeptide: methyltransferase (MTase) and restriction endonuclease (REase). Żylicz-Stachula *et al.* identified, sequenced and cloned the *taqIIIRM* gene to *Escherichia coli* (*E. coli*) bacteria. The obtained recombinant RM.TaqII enzyme recognizes only one sequence 5'-GACCGA-3' (Żylicz- Stachula A. *et al.*, 2011, 2014)

The difference in substrate specificity of the native and recombinant enzyme RM.TaqII has led to initiation of studies aimed to explain observed phenomenon. The research hypothesis undertaken and confirmed in presented thesis reveals the existence of the *taqIIIRM* orthological gene in the *T. aquaticus* YT-1 genome, encoding the RM.TaqIII protein.

In the order to verify the research hypothesis, a bioinformatic analysis of the *T. aquaticus* YT-1 genome was performed, the *taqIIIRM* gene was identified, localized and cloned to *E.coli* bacterial cells. Subsequently, the recombinant gene was expressed and recombinant enzyme RM.TaqIII was purified to functional homogeneity. The obtained enzyme preparation was used to confirm the 5'-CACCCA-3' DNA sequence recognized by REase RM.TaqIII.

Results of the study showed the validity of the research hypothesis and proved the existence of functional *taqIIIRM* gene, homologous to the *taqIIIRM*, located in one of the six megaplasmids *T. aquaticus* YT-1. The identified enzyme RM.TaqIII exhibits close similarity of the aminoacid sequence (aa) to previously characterized aa sequence of RM.TaqII (Skowron P.M. *et al.*, 2017).

