

DNA-DNA ligations with a thermostable RNA ligase from the *Thermus scotoductus* bacteriophage TS2126

Unnur Unnsteinsdóttir¹⁾, **Arnþór Ævarsson**²⁾, **Jakob K. Kristjánsson**²⁾ and **Snorri Þór Sigurðsson**¹⁾

¹⁾ Science Institute, University of Iceland, Dunhaga 3, 107 Reykjavík. unnuru@hi.is

²⁾ Prokaria, Gylfaflöt 5, 112 Reykjavík

Abstract: Nucleic acid ligases ligate two nucleic acid strands together and are extremely useful tools in molecular biology. The ligases join 5'-phosphoryl and 3'-hydroxyl ends of RNA and/or DNA oligonucleotides by a three step reaction mechanism, which requires ATP and divalent metal ions. We describe here the properties of a thermostable RNA ligase, TS2126 RNA ligase, from the bacteriophage TS2126 that infects the thermophilic bacteria *Thermus scotoductus*. We have optimized the reaction conditions for ligation of two DNA oligonucleotides and shown that the yields of ligation are good to excellent. The TS2126 RNA ligase can efficiently circularize single stranded oligonucleotides and therefore, ligation of two oligomers required strategic blocking of the 3'-end of the 5'-phosphorylated oligonucleotide. We have also investigated the effect of the oligonucleotides length and the structure of the terminal nucleotides on the 5'- and the 3'-ends on ligation yields. The effect of using a template oligonucleotide to bring two oligonucleotides closer together to enhance the efficiency of ligation was also studied. These experiments were complicated by efficient ligation of the template nucleotide to the other oligomers, in part because of unexpected phosphorylation activity. Finally, the potential of using the TS2126 RNA ligase in gene synthesis was demonstrated by sequentially ligating three oligomers together.