Hybrid Twa DNA polymerase with enhanced enzymatic activity

At the Institute of Molecular Genetics, high-fidelity DNA polymerase from *Thermococcus waiotapuensis* (Twa) was prepared, which, however, exhibited lower processivity in PCR than some other commercially available high fidelity DNA polymerases. To increase utility of the Twa polymerase, a hybrid polymerase (Twa-S) and optimized reaction buffer were prepared in the framework of TACR gama program. Twa-S polymerase exhibited higher resistance than Twa to relatively high concentrations of $(NH_4)_2SO_4$ (**Fig. 1**) and KCl (**Fig. 2**) in optimized reaction buffer. When compared to Twa enzyme or Phusion polymerase, Twa-S was capable to amplify longer DNA fragments (**Fig. 3 and 4**). All three polymerases showed comparable proof-reading activity, which was approximately 50-fold higher than the one of Taq DNA polymerase.

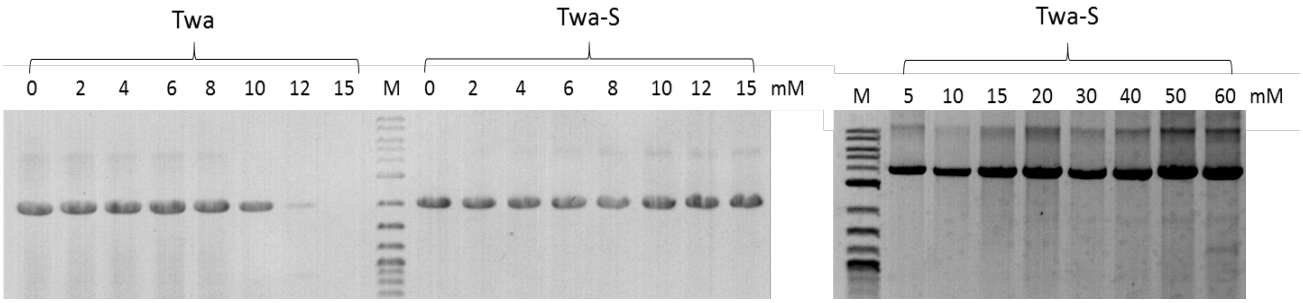


Fig. 1. Enhanced resistancy of Twa-S polymerase to relatively high concentrations of $(NH_4)_2SO_4$. Using PCR, 2 kb fragment of λ DNA was amplified with Twa or Twa-S polymerase in the presence of increasing concentrations of $(NH_4)_2SO_4$. PCR amplicons were analyzed in agarose gel in the presence of ethidium bromide. M, DNA marker. The results show that amplification with Twa-S can is not inhibited by 60 mM $(NH_4)_2SO_4$, whereas Twa parental enzyme is inhibited at 12 mM and higher concentrations of $(NH_4)_2SO_4$.

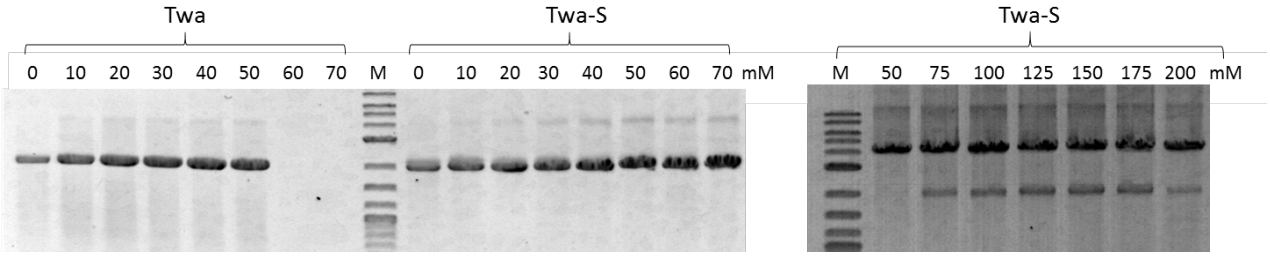


Fig. 2. Enhanced resistancy of Twa-S polymerase to relatively high concentrations of KCl. Using PCR, 2 kb fragment of λ DNA was amplified with Twa or Twa-S polymerase in the presence of increasing concentrations of KCl. PCR amplicons were analyzed in agarose gel as in Fig. 1. M, DNA marker. The results show that amplification with Twa-S is not inhibited by 200 mM KCl, whereas parental Twa enzyme is inhibited at 60 mM and higher concentrations of KCl.

Fig. 3. Twa-S polymerase exhibits higher amplification efficiency than Twa and Phusion polymerases. Using PCR, 2 - 8 kb λ DNA fragments were amplified with Twa, Twa-S or Phusion polymerases. PCR amplicons were analyzed as in Fig. 1. Each polymerization step was for 50 s. Twa-S showed higher efficiency than other polymerases when 6 and 8 kbs fragment were amplified.

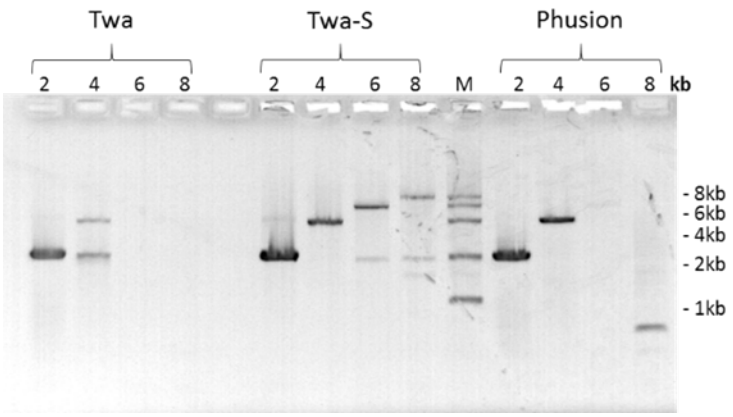
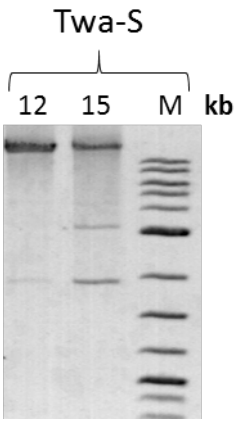


Fig. 4. Twa-S polymerase is capable to amplify DNA fragments up to 15 kb. Twa-S polymerase was used for amplification of 12 and 15 kb fragment of λ DNA in optimized reaction buffer under optimal reaction conditions. Each polymerization step was for 8 min. PCR amplicons were analyzed as described in Fig. 1.



Conclusion: The results show that Twa-S DNA polymerase exhibits new valuable properties, which predestine this enzyme for PCR amplification of long DNA fragments used for cloning.

To buy nonexclusive license for the plasmid coding Twa-S polymerase and other information regarding this project, please contact Center for Technology Transfer, IMG AS CR, Videnska 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).