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 (NH₄)₂SO₄ (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 resistance of Twa-S polymerase to relatively high concentrations of (NH₄)₂SO₄. Using PCR, 2 kb fragment of λ DNA was amplified with Twa or Twa-S polymerase in the presence of increasing concentrations of (NH₄)₂SO₄. 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 (NH₄)₂SO₄, whereas Twa parental enzyme is inhibited at 12 mM and higher concentrations of (NH₄)₂SO₄.

Fig. 2. Enhanced resistance 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, Vědecká 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).