Volume 17 Number 24 1989 Nucleic Acids Research

DNA sequencing with thermostable Tet DNA polymerase from *Thermus thermophilus*

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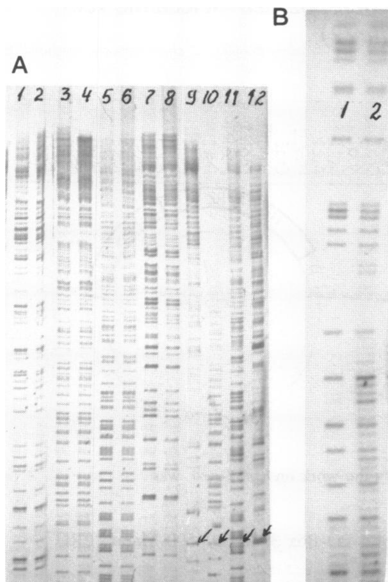
Submitted November 14, 1989

One of the modern exciting improvements in Sanger dideoxynucleotide DNA sequencing method is in the replacement of the Klenow fragment of E.coli DNA polymerase by a highly thermostable one from Thermus aquaticus (Taq polymerase) (1). Here we communicate that another thermostable enzyme from Thermus thermophilus strain KM (Tet polymerase) can be succesfully used in DNA sequencing instead of expensive Taq polymerase. Tet polymerase share common with Taq polymerase properties:absence of 3'exonuclease activity, very high processivity and wide temperature range. Tet polymerase is superior to Klenow fragment in DNA sequencing both for simplicity (the reactions take only 3 minutes at 70°C) and clear resolution of sequence ladder artefacts common for E.coli enzyme (fig.A.,arrow). Such high performance reactions are achieved at a relatively increased amount of dideoxynucleotides in mixes as compared with other polymerases including Taq polymerase. Generaly, this not diminishes the number of readable bases and we reproducibly obtain more sequence data from a single reaction than with Klenow fragment.

Fig.A. Autoradiograph of a polyacrylamide/urea gel demonstrating comparison of sequencing by Tet polemerase (lane 1 to 8) and Klenow fragment (lane 9 to 12). Single-stranded mp19 template containing 1,1 kb BglII-KpnI fragment of the LYS2 gene of Saccharomyces cerevisiae, ^{33}P-labelled (GIPCH, Leningrad) M13"forward" sequencing primer were prepared by standard methods. DNA sequencing reactions by Tet polymerase were performed in the Tet buffer containing: 20mM Tris(pH 8),10mM $MgCl_2$, 0,05% Tween 20 and Nonidet P-40. For annealing reaction 10 mkl mixture containing 5 ng of labelled primer,1mkg of template DNA in sequencing buffer was heated to 95°C for 2 min and incubated at 42°C for 10 min,cooled to room temperature.Then 1mkl (12 units) of Tet polymerase was added to this mixture and resulting mix was immediately dispensed in 2 mkl aliquots into the 4 tubes containing the 2 mkl termination mixes. The concentration of each deoxynucleotides in then were 4 mkM and dideoxynucleotides in "G"mix 160 mkM ddGTP, in "A"mix and "T"mix 500 mkM ddATP & ddTTP, "C"mix 350 mkM (lanes 1,3,5,7). Decrease the concentration of ddNTP do not lead to significance change the data picture (lanes 2,4,6,8), but in the bottom of sequence ladder we can see additional bands due to nonspecific termination (fig.B."T"mix 500 mkM & 250 mkM ddTTP, lane 1 & 2). Reaction mixtures were incubated at 72°C for 3 min and reaction stopped by the addition of 3 mkM of formamide dye solution. Samples were heated at 95°C for 3 min before loading 1-2 mkl onto a wedge 5% acrylamide gel and ran at constant power 40 W.



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