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Reverse transcription of mRNA by *Thermus aquaticus* DNA polymerase

M.D.Jones and N.S.Foulkes

Royal Postgraduate Medical School, Du Cane Road, London W12 0NN, UK
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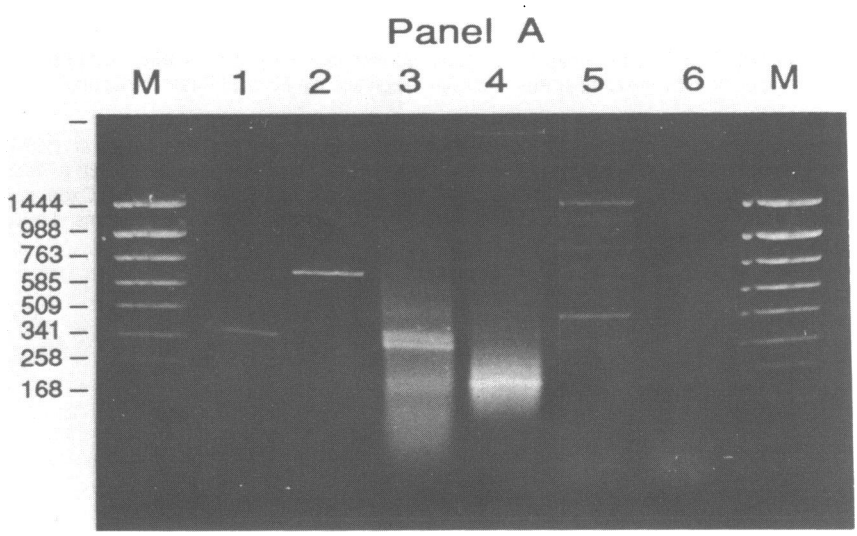
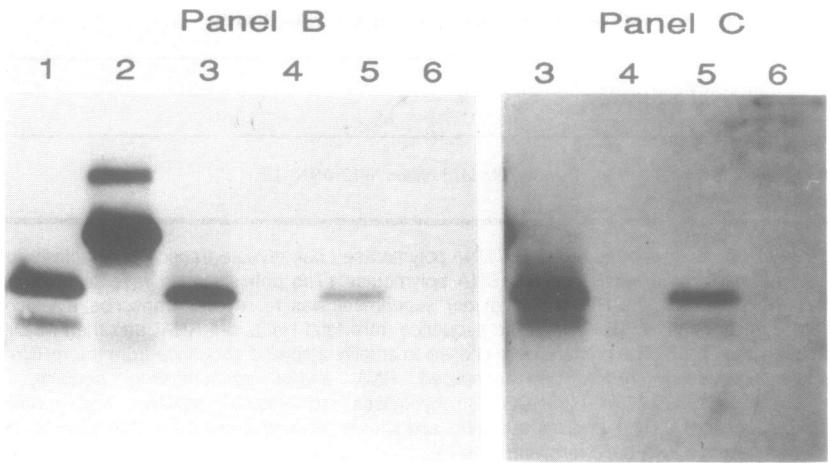
Loeb *et al.* (1) have reported that *E.coli* DNA polymerase I has reverse transcriptase activity *in vitro*. We report here that *Thermus aquaticus* DNA polymerase (*Taq* pol) also has reverse transcriptase activity at 68°C *in vitro* (2). The design of our experiment was to reverse transcribe a Glucose-6-phosphate dehydrogenase (G6PD) specific sequence from total HeLa cell RNA, and then amplify the cDNA sequence by PCR. The primers were chosen to amplify a spliced sequence from the mRNA, thus discriminating between mRNA and unspliced RNA and/or contaminating genomic DNA. Primer G (GGGAAGGAGGGTGGCCGTG) hybridises to G6PD mRNA, and primer F (CTTCAACCCCGAGGAGT) hybridises to cDNA, and should produce a spliced mRNA band of 358 bp, and a 657 bp genomic DNA band following PCR (3).

Aliquots of HeLa cell RNA (~5 µg) were treated as follows; a) with 20 units of AMV reverse transcriptase (RT) and 1 µg primer G in 20 µl of RT buffer (4) for 45 min at 42°C (see figure, lane 3); b) as for a) but with a prior incubation with 10 µg of RNase A (20 min, 37°C, lane 4); c) with 2.5 units of *Taq* pol (Perkin Elmer Cetus) and 1 µg primer G in 20 µl PCR buffer (5) for 30 min at 68°C (lane 5). After the reverse transcription steps, 1 µg of primer F and 2.5 units of *Taq* pol were added, the volume adjusted to 50 µl and the samples amplified through 30 cycles of PCR (94°C, 1 min; 55°C, 1 min; 70°C, 4 min) using a Perkin Elmer Cetus DNA Thermal Cycler. d) HeLa RNA directly amplified with 1 µg each primers G and F, and 2.5 units of *Taq* pol, in PCR buffer for 30 cycles (lane 6). Lanes 1 and 2 are a G6PD cDNA clone (10 ng) and a λ genomic clone DNA (10 ng) amplified for 30 cycles, respectively. 8 µl of the PCR products were analysed on a 1.7% agarose gel (panel A, ethidium bromide stained gel), and Southern blotted and probed with the G6PD cDNA clone (panels B and C, autoradiograph of the blot). Lanes M are size markers of an equimolar mixture of *Taq*I digested and *Sau*3AI digested Bluescribe DNA (6).





Panel A, lanes 3 and 5 show that reverse transcription of the mRNA has yielded a smear of amplified DNA. However, Southern blotting detects in both cases the spliced G6PD product (panels B and C, compare with lane 1), clearly indicating that *Taq* pol has reverse transcribed mRNA. Interestingly, RNA treated with RNase also yielded amplified DNA, although no G6PD specific band was detected. Submitting RNA directly to PCR (lane 6) did not yield any amplified DNA. In none of the RNA amplified tracks was a band equivalent to unspliced RNA or genomic DNA detected (panels B and C, compare with lane 2). We have obtained identical results using cloned *Taq* polymerase (AmpliTaq from Perkin Elmer Cetus). The reverse transcription by *Taq* pol has an optimum Mg requirement of 2-3 mM. In conclusion, the results demonstrate that *Taq* polymerase does possess reverse transcriptase activity, and could prove useful in the construction of cDNA libraries and in overcoming problems of stable secondary structures in mRNA which inhibit reverse transcription.

ACKNOWLEDGEMENTS

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References. 1. Loeb, L.A. *et al.* (1973) *Nature New Biol.*, **242**, 66-69. 2. Chien, A. *et al.* (1976) *J. Bacteriol.*, **127**, 1550-1557. 3. Persico, M.G. *et al.* (1986) *Nucleic Acids Res.*, **14**, 2511-2522 & 7822. 4. Watson, C.J. & Jackson, J.F. (1985) in *DNA Cloning, vol. 1*, (Ed. D.M. Glover) IRL Press, Oxon, pp 79-88. 5. Saiki, R.K. *et al.* (1988) *Science*, **239**, 487-491. 6. Stratagene Cloning Systems, La Jolla, California, USA.