# The use of $K^+$-free buffers eliminates a common cause of premature chain termination in PCR and PCR sequencing

**K. Woodford, M. N. Weitzmann and K. Usdin***

Section on Genomic Structure and Function, National Institute of Diabetes, Digestive and Kidney Diseases, Building 8, Room 202, National Institutes of Health, 8 CENTER DR MSC 0830, Bethesda, MD 20892-0830, USA

Received October 19, 1994; Revised and Accepted January 5, 1995

Premature chain termination at the beginning of a G-rich region is a common PCR sequencing artifact. Figure 1 shows the premature chain termination found in a region of the 33 kDa subunit of human RNA polymerase II. We have found other similar blocks to DNA synthesis in a wide variety of other sequences including the chicken β-globin promoter (1), the high copy number family of mammalian retrotransposable elements known as L1 (Howell and Usdin, manuscript in preparation), the basic fibroblast growth factor promoter and the coding sequence of the human Y-box binding protein (M. N. Weitzmann and K. Usdin, unpublished observations). While these sequences do not show sequence homology, they all contain regions that have a number of contiguous guanines separated by a small number of non-guanine bases. Templates containing such regions are also often poor substrates for PCR, showing low yields of full-length products (1). Improving template purity, using different thermostable polymerases, varying the pH and $MgCl_2$ concentration, raising the annealing temperature, or lengthening the extension step does not improve sequence quality, nor does the addition of glycerol, formamide, Gene 32 protein or single-stranded binding protein (data not shown). In addition, treatment of the reaction products with terminal transferase (2) does not improve matters when little if any chain extension past this region has occurred.

The single most effective means of reducing this artifact, is to eliminate $K^+$ from the reaction (Fig. 1). Potassium chloride is a component of most of the recommended buffers that are used in PCR and PCR sequencing reactions. This includes most commercial enzyme storage buffers, the $T_4$ polynucleotide kinase buffers used for primer end labeling, or the PCR or PCR sequencing buffers themselves. We have found that $K^+$ ions can be eliminated from all of these buffers without adversely affecting sequencing quality (Fig. 1). Removal of $K^+$ is effective since it presumably eliminates or greatly reduces the stability of alternate DNA structures that can form in G-rich regions (1,3). Any residual premature chain terminations can then be eliminated by treatment with terminal transferase if necessary.

This simple modification to existing protocols may facilitate the PCR or PCR sequencing of G-rich regions that are otherwise difficult to sequence or to amplify. We have also found that in general, the presence of even small amounts of monovalent cation of any sort adversely affects sequence quality, causing frequent, albeit milder, chain termination at many places on the template (see Fig. 1). The routine use of monovalent cation-free buffers should improve productivity in large scale sequencing projects.

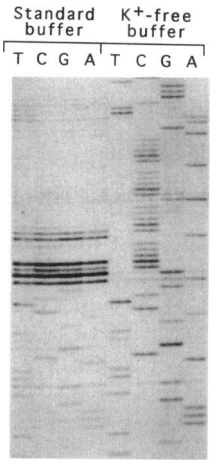


**Figure 1.** Linear amplification sequencing of part of the G-rich motif found in the gene for the 33 kDa subunit of human RNA polymerase II (4). The G-rich region was cloned in the plasmid pMΔ189 as described elsewhere (1). The primer used was SupFR4 (5′-ATGCTTTTACTGGCCTGCT-3′). Two and a half units of *Taq* polymerase (Gibco-BRL, Gaithersburg, MD, USA) was added to a reaction mix containing 500 fmol of template, 15 pmol of unlabeled primer, 10 μCi of [α-$^{35}$S]dATP (1000 Ci/mmol), 80 mM Tris–HCl (pH 9.3) and 4 mM $MgCl_2$. Four microliter aliquots of this mixture were then added to 2 μl of a termination mix containing 15 μM each of dATP, dCTP, dTTP and 7-deaza-dGTP and either 0.03 mM of ddGTP or ddCTP, 0.45 mM ddATP or 0.9 mM ddTTP. Each reaction was overlayed with a drop of mineral oil and the reactions cycled 30 times for 30 s at 94°C, 30 s at 55°C and 30 s at 72°C. The reactions were stopped by the addition of 3 μl of Stop buffer containing 95% (v/v) formamide, 10 mM EDTA (pH 7.6), 0.1% xylene cyanol and 0.1% bromophenol blue. The samples were heated to 90°C for 2 min and loaded on a 5.5% sequencing gel.

## REFERENCES

1 Woodford,K., Howell,R. and Usdin,K. (1994) *J. Biol. Chem.* **269**, 27029–27035.
2 Fawcett,T.W. and Bartlett,G. (1990) *Biotechniques* **9**, 46–48.
3 Sen,D. and Gilbert,W. (1990) *Nature* **344**, 410–414.
4 Pati,U.K. and Weissman,S.M. (1990) *J Biol. Chem.* **265**, 8400–8403.

* To whom correspondence should be addressed