4220–4221 Nucleic Acids Research, 1995, Vol. 23, No. 20

PCR product with strands of unequal length

Kelly P. Williams and David P. Bartel*

Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA

Received September 6, 1995; Accepted September 8, 1995

Biochemical properties of ssDNA molecules compare interestingly with those of RNA; ssDNAs capable of binding target molecules (1–3) or catalyzing reactions (4–5) have been isolated from synthetic pools of random-sequence ssDNAs by repeated cycles of *in vitro* selection and PCR amplification. The negative (–) strands of the PCR product, while necessary for amplification, can interfere with binding or catalytic activities of positive (+) strands. One method for reducing this interference has been to use asymmetric PCR to generate more (+) strands than (–) strands (2,3). Ideally, all (–) strands would be removed from an amplified (+) ssDNA pool. Toward this goal, specific biotinylation of the (–) strand followed by streptavidin separation has been used (1,4,5), and an approach involving a detachable primer has recently been described (6). Biotin-based ssDNA preparation may be undesirable if another biotin-based separation is planned during a different step of the *in vitro* selection protocol. Here we present a simple method for purification of the (+) PCR product strands that involves incorporating a terminator of (+)-strand synthesis within the (–)-strand PCR primer, causing the two product strands to differ significantly in length (Fig. 1). The (+) ssDNA can then be readily purified by gel electrophoresis. The (–) ss DNA can also be saved and used to regenerate the complete pool.

The (–)-strand PCR primer has a tripartite composition: 5′-lengthener-terminator-complement (Fig. 1A). The complement segment serves the priming function, the lengthener segment is responsible for the size difference of the two strands, and the intervening terminator is non-nucleotide material that blocks (+)-strand elongation. The terminator is composed of two successive triethyleneglycol phosphate units, incorporated using the Glen Research spacer phosphoramidite 9. The sequence of the flanking DNA was designed to enhance terminator function. The lengthener sequence is poly-dA, and a purine is excluded at the 5′ position of the complement segment so as to prevent the terminal 3′ nucleotide of the (+) strand [either the legitimate templated terminal 3′ nucleotide or the non-templated 3′ adenosine residue frequently added by *Taq* DNA polymerase (7)] from bridging the terminator to pair with the lengthener and prime continued extension.

Efficacy of the 'primer-terminator' is illustrated by strand-specific labeling of PCR products, and analysis by denaturing gel electrophoresis (Fig. 1B). The (–) strands (lane 1) migrate much more slowly than do the (+) strands (lane 2) and terminator readthrough is only 0.3%. A slice sufficiently broad to include most anomalously migrating (+) strands (barred zone of lane 1) contains <2% of the radioactivity of the full-length (–) strand.

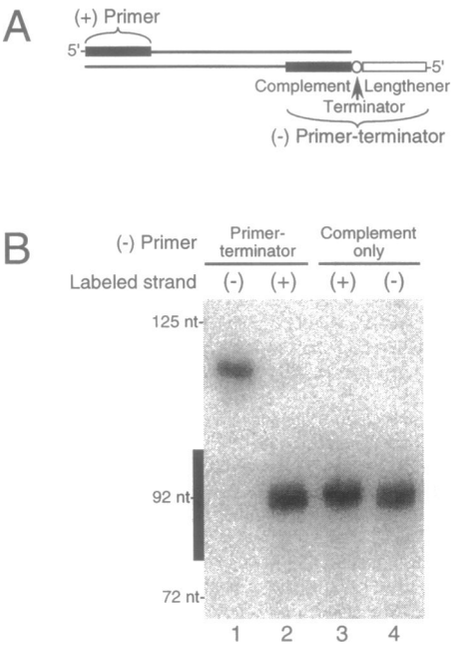

Figure 1. Unequal strand length in PCR using a primer-terminator. (**A**) Schematic of PCR product. The (–)-strand primer-terminator is interrupted by spacer material (terminator) that prevents (+)-strand elongation. (**B**) Strand separation in a denaturing gel. The (–)-strand primer was either primer-terminator (lanes 1 and 2) or normal (lanes 3 and 4). Positions of markers (filled-in *Msp*I digest of pBR322 DNA) are indicated; 86–100 nt DNA would migrate within the zone indicated by the bar.

Further purification could be based on the unpaired lengthener (poly-dA) portion of the (–) strands.

The size of the lengthener segment can be tailored to the length of the pool molecules to be amplified. In the experiment of Figure 1, a primer-terminator with a 20 nt lengthener was used to amplify a 91 nt ssDNA pool. Similar success has been obtained using a primer-terminator with a 35 nt lengthener to convert a dsDNA pool (9) into a 274 nt (+) ssDNA pool.

PCR and analysis. Mixtures of 1 μM each primer and 3 nM template in 100 μl 10 mM Tris-Cl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM dNTPs 2.5 U *Taq* DNA polymerase underwent 30 cycles of 95°C, 60 s; 46°C, 30 s; 72°C, 60 s. The template (8) was 5′-AGATTGCACTTACTATCT-$(N)_{55}$-ATTGAATAAGCTGGTAT (N indicates any of the four

* To whom correspondence should be addressed

deoxynucleotides with approximately equal probability). The (+) primer was 5′-AGATTGCACTTACTATCT. The (–) primer was either primer-terminator, 5′-$(A)_{20}$-XXTTACCAGCTTATTCAATT (X indicates triethyleneglycol phosphate), or the complement segment alone, 5′-ATACCAGCTTATTCAATT. The template and primer-terminator oligonucleotides were gel-purified; other primers were purified by butanol extraction (10). Either the (+) or (–) strand was labeled using the appropriate 5′-[^{32}P]-primer. Samples were analyzed by electrophoresis in a 10% polyacrylamide–8.3 M urea gel, and a phosphorimager was used for imaging and quantitation.

ACKNOWLEDGEMENT

This work was supported by a grant from the Keck Foundation to the Whitehead Fellows program.

REFERENCES

1 Bock,L.C., Griffin,L., Latham,J.A., Vermaas,E.H. and Toole,J.J. (1992) *Nature* **355**, 564–566.
2 Ellington,A.D. and Szostak,J.W. (1992) *Nature* **355**, 850–852.
3 Huizenga,D.E. and Szostak,J.W. (1995) *Biochemistry* **34**, 656–665.
4 Breaker,R.R. and Joyce,G.F. (1994) *Chem. Biol.* **1**, 223–229.
5 Cuenoud,B. and Szostak,J.W. (1995) *Nature* **375**, 611–614.
6 Silveira,M.H. and Orgel,L.E. (1995) *Nucleic Acids Res.* **23**, 1083–1084.
7 Clark,J.M. (1988) *Nucleic Acids Res.* **16**, 9677–9686.
8 Crameri,A. and Stemmer,W.P.C. (1993) *Nucleic Acids Res.* **21**, 4410.
9 Bartel,D.P. and Szostak,J.W. (1993) *Science* **261**, 1411–1418.
10 Sawadogo,M and Van Dyke,M.W. (1991) *Nucleic Acids Res.* **19**, 674.