Biphasic amplification of very dilute DNA samples via 'booster' PCR

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PCR amplification of fewer than 1000 copies of template is hampered by synthesis of so-called primer dimers and other spurious products. These artifacts consume the stock of primers and occupy enzyme, with consequent reduction in yield of target. To overcome this problem, we have devised Booster PCR, a biphasic amplification strategy. During Stage I, primers are diluted to obtain an initial 10^7 -fold molar excess of primer to template. At the beginning of Stage II, primer concentration is brought up to $0.1 \mu\text{M}$. This procedure differs from standard PCR methods which use 0.1 - $1.0 \mu\text{M}$ primers from the outset.

The figure compares products from standard ("S") and Booster ("B") PCR in which amplification started from very dilute human genomic DNA. In contrast to control ("C") PCR from 3×10^5 copies ($1 \mu\text{g}$ DNA), non-target amplified products are common when starting with a low copy number. (Product [*] is homologous to target.) For 250 copies of template (750 pg DNA), "S" artifacts are predominantly primer dimers (PD) whereas "B" artifacts are of higher molecular weight. The yield of target (T) is somewhat higher in "B". At 50 copies (150 pg), "B" clearly enhanced yield whereas "S" is still impaired by primer dimers. At 5-10 copies (about 20 pg DNA), the stochastic nature of Booster PCR is most evident (cf. Ref. 1). On average, yield of target is substantially higher in "B" than in "S". We attribute variation in yield to stochastic number of targets and primers "catching" them at different cycles.

Booster PCR amplifies more target because it reduces the deterministic formation of primer dimers; its artifacts could be further diminished by introducing nested primers (Ref. 2) in Stage II or by varying $[\text{Mg}^{++}]$ and Taq polymerase. When extended to a single molecule of template, Booster PCR seems to minimize the possibility that the target may be missed altogether.

Figure 1: COMPARISON OF BOOSTER ("B") AND STANDARD ("S") PCR. A 770 bp inter-Alu segment between $\psi\delta$ and δ globin genes was amplified with 5'- AAGTGGAAAGAATAGCATCT -3' and 5'- GAATGTGAATTTGTAGAAGG -3' (Ref. 3). **STAGE I of "B"**: primers and template diluted in same proportion, 83 pM primers for 250 copies, 17 pM for 50 copies, 2.5 pM for 5-10 copies; 20 cycles, 94° denaturation (1 min), 45° annealing (4 min), 72° polymerization (3 min). **STAGE II of "B"**: primers brought up to $0.1 \mu\text{M}$; 50 further cycles but annealing for 2 min. "S": same copy number as "B" but with $0.1 \mu\text{M}$ primers from outset; 50 cycles as in Stage II of "B". PCR mixes and electrophoresis as in Ref. 1. Photograph is ethidium stain of 10% of total product. Additional 20 cycles for "S" gave same result as 50 cycles. Little product is discernible for "B" if Stage II is only 30 cycles.

REFERENCES: 1. Saiki et al 1988, *Science* 239: 487; 2. Mullis and Faloona 1987, *Methods Enzymol* 155: 335; 3. Maeda et al 1988, *Mol Biol Evol* 5: 1.

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