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Improved yields of long PCR products using gene 32 protein

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Submitted December 11, 1989

The polymerase chain reaction (PCR) has recently evolved as one of the most powerful tools in molecular biology (1). The length-limits of the amplifiable target sequences are still undetermined. Since we are interested in obtaining reliable products in the range of 3–6.5 kb, we tested the effect of the gene 32 protein (gp 32) of the phage T_4, which enhances T_4 DNA polymerase activity and accuracy (3). Furthermore gp 32 was shown to improve double stranded sequencing results, no matter whether the Klenow fragment of DNA polymerase I or the T_7 DNA polymerase was used (4).

In fact, our data demonstrate that the use of 0.5–1 μl (0.5–1 nmol) gp 32 in amplification protocols applying Taq polymerase improved the yield of long amplified DNA fragments reliably at least tenfold (Fig. 1 A, B).

MATERIALS AND METHODS

– Phage J 1.1.1. DNA, which included in EMBL 3 a 16 kb BamHI genomic fragment starting 5′ of D_{HQ52} and ending 3′ of the membrane region on the human immunoglobulin heavy chain locus.

– Oligonucleotides:

KS 2: 5′ ACC CAG CAC TGG TGG ACA C 3′
KSR2: 5′ CCA ATC ATT ACC ACC CTC C 3′
KS 3: 5′ GAC GGA TCC TTC AGT GGG ACG ACG GTG AAC 3′
KSR5: 5′ GCC GTC GAC GGC AGT AGC AGA AAA CAA 3′.

– gp 32 (Pharmacia) 1 nmol/μl.
– AmpliTaq (Cetus).
– dNTPs (Pharmacia): dATP, dCTP, dGTP, TTP, 2.5 mM each.
– 10×Taq polymerase buffer: 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin.

Water (ad 100 μl), 10 μl 10×Taq buffer, 8μl dNTPs and 1 μl gp 32 were mixed. The respective oligonucleotides (50 pmol each) and DNA were added. The reaction mix was overlaid with 100 μl mineral oil. The DNA was denatured for 5 minutes at 95°C, 1.5 units of Taq polymerase were added and the amplification was started (2 minutes 95°C, 1.5 minutes 56°C and 5 minutes 70°C). After 30 cycles the DNA was extracted with an equal volume of chloroform and analysed on 0.8% agarose gels, followed by ethidium bromide staining and/or Southern blotting. As probe we used a J_H locus fragment.

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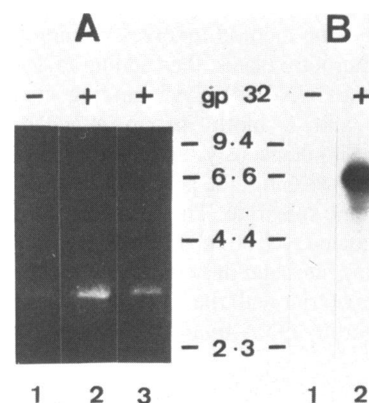

Figure 1. Analysis of 10% of the amplification products: A: Oligos KS 3/KSR5 amplified a 3 kb DNA fragment, visualized on an EtBr stained gel; B: Oligos KS 2/KSR2 amplified a 6.5 kb DNA fragment, detected after a Southern blot and hybridisation with the J_H probe. The amplification was done without (–) or with (+) 1 nmol of gp 32. DNA concentration of J 1.1.1. was A: lane 1 and 2: 100 ng/ml, lane 3: 1 ng/ml and B: lane 1 and 2: 100 ng/ml. Standard: lambda/HindIII.