A procedure for *in vitro* amplification of DNA segments that lie outside the boundaries of known sequences

Tony Triglia, M.Gregory Peterson and David J.Kemp

The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia
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The polymerase chain reaction (PCR) system allows the amplification of DNA segments between two regions of known sequence [1]. We describe a procedure that extends this technique to sequences that lie outside the boundaries of known sequences. The approach simply requires inversion of the sequence of interest by circularization and re-opening at a different site. Figure 1 shows the procedure for amplifying segments (X and Z) in a genome, adjacent to a segment (Y-Y') of known sequence. The genome is first cleaved with any restriction endonuclease (N and N' in Fig. 1) with conveniently located sites. The diluted DNA (≤ 3 μg/ml) is then ligated overnight. The circles are then cleaved at any site located between Y and Y'. Regions X and Z can be amplified using oligonucleotide primers corresponding to regions Y and Y', synthesized in opposite orientation to that for normal PCR because they have been inverted and now flank regions X and Z (Fig. 1). We therefore designate the approach "inverted PCR" (IPCR). The point at which the two ends were ligated is defined by the site N. To test this procedure we used the gene encoding the precursor to the major merozoite surface antigens of P.falciparum [2]. Chromosomal DNA (2μg) was cut with Rsa1, ligated, re-cut with Hinf1 and amplified. The expected 297 bp fragment was obtained (Fig2A&B) and checked by sequencing nine clones. It is not necessary to cleave the circles with restriction endonuclease M as the same effect can be achieved by introducing nicks by heating (Fig.2C). Many other variations of IPCR could obviously be employed. Sites could be included in the oligonucleotides to facilitate cloning. Instead of a single enzyme, two different enzymes could be used, with end-filling if necessary. Instead of cleavage with N, the DNA could be randomly sheared and then cut at a single site within the known sequence. IPCR could allow "chromosome crawling" and remove the need to generate clones for some regions of the genome altogether. This approach should be useful for completing the sequences of coding regions from mRNA's. Where a full-length cDNA clone has been obtained it would be useful for walking along genomic DNA into the promoter region. DNA flanking viral integration sites could also be sequenced.

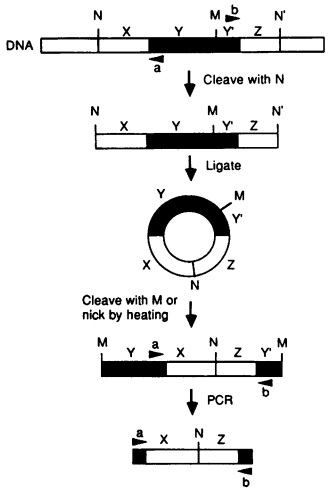

Fig.1. Amplification of sequences by IPCR. a and b are primers, other symbols are defined in the text.

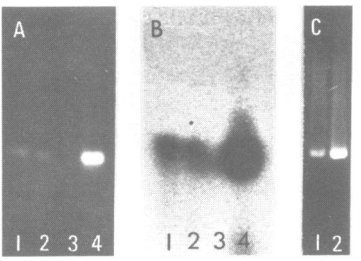

Fig.2. Amplification of the test segment from P.falciparum isolate FC27 (2). A and C, EtBr-stained DNA. B, hybridization with an oligonucleotide corresponding to region X in Fig. 1. In A and B, tracks 1 and 2 are the product from IPCR of FC27 chromosomal DNA; Track 3, no sample; Track 4, the product from IPCR of the test plasmid. In C, the circularized DNA was cut with Hinf1 before amplification (track 1), or nicks were introduced by heating 94° for 30' before amplification (track 2).

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References

1. R.K. Saiki et al., Science 239, 487-491 (1987). 2. M.G. Peterson et al., Mol. Biochem. Parasitol. 27, 291-302 (1988).

