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PCR amplification of megabase DNA with tagged random primers (T-PCR)

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A very sensitive and specific method for the random amplification of whole DNA molecules and genomes ranging from 400 base pairs (bp) to 40 Megabase (Mb) is described. This simple, two step PCR (1–3) strategy utilizes tagged random primers that consist of a pool of all possible 3′ sequences for binding to the target DNA and a constant 5′ region for the detection of incorporated primers. As little as 10^{-12} g of DNA was readily amplified to amounts that could be visualized on ethidium bromide stained gels. The high specificity of this method was demonstrated by hybridization of PCR products to gridded whole genome cosmid libraries. Tagged random primer PCR (T-PCR) is very useful for the amplification of DNA samples purified by various electrophoresis techniques or flow sorting. This random whole DNA and genome amplification should simplify the analysis of other samples with very little DNA, like individual sperm and oocytes. It has the potential for amplifying any DNA molecule.

Tagged random primers (4) containing a 9 to 15 bp arbitrary 3′ tail that can bind to any DNA sequence and a constant 17 bp 5′ head for the subsequent detection of the incorporated primer were synthesized. In the first PCR step, consisting of two or more cycles, the random 3′ part of the primer is annealed to the complementary target DNA, and target specific sequences are added by the polymerase. In the second cycle of this step, molecules containing two random primers at their ends are generated. Unbound primer and primer–primer complexes are subsequently removed by spin column gel filtration at 1100 g for 10 minutes with Biogel P100 (5). During the second PCR step, molecules that have incorporated two random primer are amplified *ad libitum* with a single primer complementary to the constant 5′ part of the tagged primer. The annealing conditions of the first step PCR were determined by the length of the random part of the primer. For example, an annealing temperature of 30°C for 1 minute was used for a primer with 9 random bases. The temperature was then raised to 40°C for 1 minute and at 72°C for 2 minutes for polymerization. Samples were denatured for 30 seconds at 96°C at the beginning of each PCR cycle. For the first step PCR the primer concentration was adjusted to the sample DNA concentration (see below). For the second step PCR the primer was added to a final concentration of 2.5 μM. The standard PCR buffer contained 4 mM $MgCl_2$ and 100 μM of all four dNTPs.

In pilot studies collections of all possible 10 to 20 bp random primers were used for the amplification of several DNA species. In most cases, 60 PCR cycles (two consecutive 30 cycle PCR's) were necessary to detect amplified products which appeared as a smear on agarose gels. The smear indicated the presence of amplified DNA molecules, but did not, *per se*, prove the amplification of target sequences. Since the random primers were present during the entire PCR procedure, primer–primer extension could not be ruled out. To examine the specificity of the random PCR, products of such amplifications of *Schizosaccharomyces pombe* chromosomal DNA and macrorestriction fragments were hybridized to whole genome cosmid libraries. The results were compared with hybridizations of probes purified by preparative electrophoresis techniques, as demonstrated for *S.pombe* chromosome I DNA in Figure 1A and C. In general, the random PCR products hybridized to all cosmid clones of the genome, indicating the amplification of unspecific DNA sequences (Figure 1C). Only a small number of clones that contained repetitive DNA sequences showed stronger hybridization signals. Hence, most of the DNA amplified by the random primers PCR did not originate from the template DNA, but is the result of primer–primer extensions.

These results motivated our development of the T-PCR method. DNA samples ranging in size from 400 bp up to 40 Mb were tested (Figure 2). Most amplified products of megabase DNA ranged in size from about 400 bp to 1.6 kb. DNA samples from ethidium bromide stained gels with prior UV exposure showed somewhat smaller products compared to unirradiated samples. Hybridizations of T-PCR products to gridded whole genome cosmid libraries were almost identical to results obtained by the original pure probe, as demonstrated for *S.pombe* chromosome I DNA in Figure 1A and B. Similar results were obtained with pools of small *S.pombe* fragments, *S.pombe* macrorestriction fragments and human chromosome 21 DNA. There was no evidence for the presence of any nonspecific DNA. Slight differences in some signal intensities were probably due to the random distribution of primers along the target DNA and the sequence and fragment size dependent amplification bias of the *Taq* polymerase. Repetitive *S.pombe* sequences and presumably human repetitive sequences such as Alu elements were not amplified with any preference.T-PCR using primers containing 12 or more random bases showed weak hybridization to all colonies, indicating the amplification of nonspecific sequences. This is presumably due to the less efficient elimination of these longer primer during the gel filtration step.

The mass ratio of primer to DNA affected the yield and the

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size distribution of the T-PCR products. In general, a wide range of primer−DNA mass ratios was suitable for T-PCR amplification. However, the yield of amplified products dropped significantly with lower amounts of primer while the mean product size increased. High primer concentrations produced smaller fragments. A random primer to DNA mass ratio of 30 to 50 appeared to be most suitable for T-PCR. The sensitivity of T-PCR was tested on decreasing amounts of DNA. As little as 10^{-12} g DNA, equivalent to approximately two copies of human chromosome 21, was readily amplified with 35 PCR cycles and could be visualized on ethidium bromide stained gels. Increased cycle numbers resulted in even higher sensitivity, but may not be necessary for most practical purposes. Excessive cycling may also have the disadvantage of generating elevated levels of nonspecific PCR products (6).

Up to now, two methods have been described for the amplification of genomic regions or whole genomes. In the first method, specific linkers were ligated to the ends of DNA fragments (7, 8). DNA was subsequently amplified with primer complementary to the linker. This ligation mediated approach has the potential of amplifying DNA *ad infinitum*. It needs, however, a considerable amount of sample manipulation which may lead to the loss of portions of the genome in case of single cell analysis (9). The random distribution of restriction sites may also generate fragments that are too small or too long for PCR amplification. Ligated primer−primer complexes can produce nonspecific PCR products. The second method used partially or completely degenerate oligomers for the amplification of DNA (9, 10). Random 15-mers reportedly did not amplify single cell DNA up to amounts that could be detected on ethidium bromide stained gels (50 PCR cycles) (9). Specific sequences were detected with an additional PCR with pairs of specific primers. This random primer strategy was identical to one of the initial approaches we tried. However, the two major problems mentioned above finally led us to abandon this strategy in favour of the T-PCR procedure. On the other hand, degenerate oligonucleotide-primed PCR (DOP-PCR) demonstrated efficient genome amplification of various species (10). However, the specificity and complete genome coverage of this amplification technique needs to be proven.

In contrast, T-PCR combines the advantage of incorporating any DNA sequence by the random primer technique with the amplification rate of standard two primer PCR. Products were amplified at exponential rates with high specificity. Fragments as small as hundreds of base pairs as well as entire genomes can be readily amplified by this technique. Hybridizations of such amplified products were almost identi cal to results obtained from the original probes. These results indicate that tagged random primers can be used for the amplification of almost any DNA molecules. The high sensitivity makes T-PCR suitable for the amplification of small amounts of DNA such as samples consisting of only single cells.

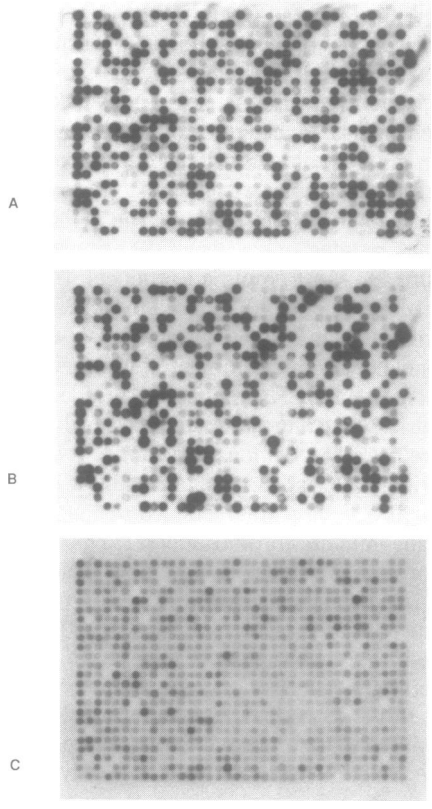

Figure 1. Specificity of random primer PCR. Probes were hybridized to the same filter of a gridded *S.pombe* cosmid library. **A**: *S.pombe* chromosome I DNA purified by preparative PFG. **B**: T-PCR: *S.pombe* chromosome I DNA amplified with tagged primer with 9 random bases (35 cycles). **C**: Random primer PCR: *S.pombe* chromosome I DNA amplified with a collection of all possible 9-mers.

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REFERENCES

1. Saiki,R., *et al.* (1985) *Science* **230**, 1350−1354.
2. Li,H., *et al.* (1988) *Nature* (London) **335**, 414−417.
3. Jeffreys,A.J., *et al.* (1988) *Nucl. Acids Res.* **16**, 10953−10971.
4. Jeffreys,A.J., *et al.* (1991) *Nature* (London) **354**, 204−209.
5. Sambrook,J., *et al.* (1989) Molecular Cloning: A Laboratory Manual. Second Edition. Cold Spring Harbor University Press, Cold Spring Harbor, N.Y.
6. Bell,D.A. and Demarini,D.M. (1991) *Nucl. Acids Res.* **18**, 5079.
7. Lüdecke,H.-J., *et al.* (1989) *Nature* (London) **338**, 348−350.
8. Kinzler,K. and Vogelstein,B. (1989) *Nucl. Acids Res.* **17**, 3645−3653.
9. Zhang,L., *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5847−5851.
10. Telenius,H., *et al.* (1992) *Genomics* **13**, 718−725.

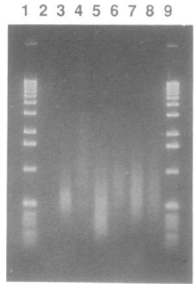

Figure 2. T-PCR amplification of various DNA samples. Samples are: 1 kb ladder size standard (Gibco BRL), lanes 1 and 9; control, no DNA added, lane 2; *S.pombe HindIII* fragments (400 to 500 bp), lane 3; pBR 328, lane 4; *S.pombe SfiI* fragment F, lane 5; *S.pombe* chromosome 1, lane 6; *S.pombe*, whole genome, lane 7; flow sorted human chromsome 21 DNA, lane 8. With the exception of sample lane 5, primers were removed by Biogel P100 gel filtration. *S.pombe SfiI* fragment F DNA was purified less efficiently with Sephadex G50 (LKB-Pharmacia).