The polymerase chain reaction (PCR) has become the most widely used technique for the manipulation of DNA with applications for cDNA cloning, gene cloning, polymorphism detection, mutagenesis and allele-specific diagnosis (8). All of those applications have been extended significantly by the recent development of “long” PCR, a technique that makes it possible to amplify DNA fragments up to 40 kb in length (2,3). Included among important factors for practical use of the PCR are high specificity and high yield. Many different approaches have been used in an attempt to increase both reaction yield and specificity. For example, considerable effort has been expended on the optimal design of PCR primers and on modifications of reaction conditions, such as the use of a so-called “hot start”, the initial addition of reagents at an elevated temperature (4,5).

Nonspecific amplification is a particular problem in PCR when it is not possible to design optimal primers, either as a result of limited knowledge of the DNA sequence to be amplified or in situations in which multiple copies of similar sequences are present in the template DNA. We have recently attempted to deal with such a situation by using an approach that we term “selective suppression” by restriction endonuclease digestion. That approach combines, in a single reaction tube, restriction endonuclease digestion and a hot start for PCR. This technique eliminates template DNA sequences that may result in nonspecific amplification by restriction digestion performed immediately before PCR; so it is unnecessary to design highly specific primers. Furthermore, since template sequences that may compete during PCR have been removed before the reaction, it is also possible to obtain increased yields of the desired amplification product. Reagents required for restriction digestion of template DNA are initially separated from the PCR reactants by a wax barrier. However, after hot start of PCR, final concentrations of all reactants are optimal for performance of PCR.

Selective suppression of long PCR will be illustrated by its use to amplify individual members of a group of at least three human chromosome 16 phenol sulfotransferase (PST) genes, STP1, STP2 and STM (1,7). With the exception of regions that contain Alu sequences, these three genes display very high sequence homology, even within introns (1,7). A major problem associated with the use of PCR to amplify these three genes with genomic DNA as a template has been the nonspecific amplification of sequence fragments from more than one gene. The use of selective suppression makes possible higher yields of specific amplification products by initial restriction digestion that leaves only one of the PST gene sequences intact, which is followed by performance of long PCR in the same reaction tube. When long PCR (GeneAmp®XL PCR Kit; Perkin-Elmer, Norwalk, CT, USA) is performed with human genomic DNA as a template, segments from all three PST genes are amplified simultaneously by primers F2 (5′-AATGCCCACGCA-AGTGCCCTGCTGAG-3′) and R2 (5′-CTCCGCTAGTCCGACATCG-3′).
amplification specificity and yield of Eco

eestion, in this case with expression by restriction endonuclease di-

human PST multigene system to study genes as a template. We have used this only

should leave only long PCR, while digestion with

and R2 are summarized in Table 1. The

table shows that, in theory, digestion of STM

characters for the

but not STM. Selected restriction character-

primers F2 and R2 are approximately

STP1

to include nearly all of the coding ex-

flanking regions of the respective genes

sequence that extends from the 5′

restriction enzyme (0–8 U) in a 22-

fering quantities of restriction enzyme

upper reaction mixture contained dif-

for 20 min during the restriction en-

PCR tubes over the wax barrier. The

reaction mixture was then added to the

480 DNA Thermal Cycler. An upper

STM

obtained with

Eco

RI (B) before amplification reaction, but in the same reaction tube.

obtain this lower reaction mixture, to-

STM

and

Eco

RI will digest

I sites, but

STM

STP2

but not

STM

Eco

RI, before long PCR to enhance

Figure 1. Selective suppression of long PCR by restriction digestion of human genomic DNA with

XbaI (A) or EcoRI (B) before amplification reaction, but in the same reaction tube. A 7.5-kb partial

human STP2 fragment as well as 7.0-kb STM and 6.9-kb STP1 fragments are indicated by arrows. The

figure shows ethidium bromide-stained 1% agarose gel electrophoresis of 10 µL of long PCR mixtures.

Specifically, PCR was performed with a 78-µL reaction mixture that in-
specific PCR primers for very similar sequences. It is particularly useful in situations in which limited sequence information is available for primer design for at least one of the fragments that is to be amplified. Selective suppression can also be used to specifically amplify individual members of a variety of highly homologous gene families using DNA obtained in the course of population studies of genetic polymorphisms—in essence, using similarity of a sequence as an advantage rather than as an obstacle. Finally, the selective suppression technique will potentially be even easier to apply when thermostable restriction endonucleases become widely available (10).

REFERENCES


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Simplified Protocol of Solid-Phase cDNA Libraries for Multiple PCR Amplification


Reverse transcription (RT) followed by polymerase chain reaction (PCR) amplification is a widely used, highly sensitive method to analyze the expression of low abundance messenger RNAs extracted from very small samples (down to one or a few cells). Nucleic acids can also be extracted from limited tissue fragments on slides using microdissection technology (5). In these cases, the number of mRNAs amplified with PCR technology is reduced, even when using multiplex PCR amplification, and then the detection of multiple mRNAs requires multiple sampling. This limiting factor becomes a very important problem; for instance, when the phenotype of a well-delimited cellular group must be precisely determined, or when the sample contains various intermingled cell populations of interest to be characterized.

The use of magnetic beads technology is well adapted to the extraction of small quantities of mRNA and facilitates the production of solid-phase cDNA libraries (2,6). Protocols described for subsequent PCR recommend removal of the beads after the second-strand cDNA synthesis (6). Here we report the use of immobilized cDNA libraries for multiple amplifications with beads present in the PCR tube. The main advantages of this method are (i) simplicity, (ii) economic and time savings, one extraction allowing the amplification of several genes, and (iii) convenient cDNAs storage.

We have developed this method in order to study genes expressed in a very limited region of the rat lateral hypothalamus. Adult male Sprague-Dawley rats (IFFA, Credo, France) were used for this study and killed by decapitation. Brains were immediately dissected and frozen in liquid nitrogen without a fixation step. They were cryostat-sectioned into 20-µm-thick sections thaw-mounted on glass slides. Slides were kept at -20°C until use. Tissue samples...