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Increasing Detection of Polymerase Chain Reaction (PCR) by Isolation of PCR Products (IPCRp)

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Aim To develop a method for enhanced polymerase chain reaction (PCR) product detection.

Methods During the PCR, the double-stranded product is generated with fluorescent dye on one strand, and biotin on the other strand. The product is captured on the streptavidin-coated plates with high efficiency (IPCRp). Washing of the all unamplified compounds, including dye-labeled unincorporated primers, follows the PCR. The targeted dye-labeled PCR product is released by denaturation and loaded on the detection platform.

Results After the application of the IPCRp, the resulting product is highly concentrated targeted dye-labeled single-stranded DNA, free of the unincorporated primers and other PCR artifacts. The strength of the signal of the IPCRp product on detection platform is two- to five-fold higher than the strength of the signal of the conventional PCR product.

Conclusion The IPCRp procedure can be accomplished in less than 20 minutes. Efficient isolation of the PCR products has two steps, washing and denaturation. It can increase the yield of targeted PCR product and increase the sensitivity of the detection platform.

Amplification of DNA and determination of the genotype at a given locus can be achieved by polymerase chain reaction (PCR) (1). The PCR is highly sensitive method that can have very important application when the DNA quantity is limited (2,3). This includes forensics, prenatal disease diagnosis, archeological studies, studies of ancient DNA, and other evolutionary applications. The standard PCR reaction can be efficiently used with one nanogram to one microgram of genomic DNA. However, very often in practice, there is a need to amplify template DNA that is bellow one nanogram. This is the case with amplification of low-copy number DNA and highly degraded DNA (4). Because the effect of degradation is to lower the number of amplifiable copies, the low-copy number DNA and the degraded DNA are in effect representing the same problem. Designing PCR primers close to the targeted regions is one solution to the problem of amplification of the degraded DNA. In effect, this amplifies low molecular weight DNA, which is less likely to be degraded (5,6). Also, improving the extraction efficiency can facilitate the amplification of the degraded DNA because more efficient extraction will yield higher quantities of DNA (7). The post-PCR sample preparation can also improve the detection of the degraded DNA.

Here we describe a method for the isolation of PCR products (Isolation of PCR products, IPCRp), that can increase the sensitivity of an instrument platform and enhance the detection of low-copy number or degraded DNA.

Material and Methods

Schematic diagram of the IPCRp procedure is presented in Figure 1. The method utilizes

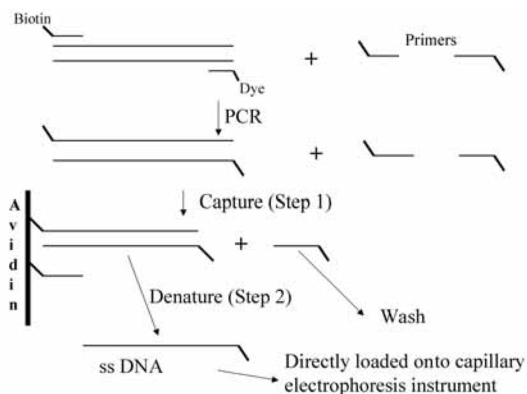

Figure 1. Schematic diagram of the isolation of polymerase chain reaction (PCR) products (IPCRp).

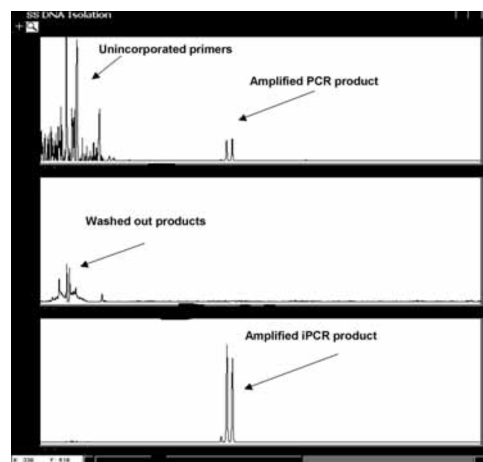

Figure 2. Amplified product after the PCR (upper panel) and after the isolation of PCR products (IPCRp, lower panel). Washed out product is shown in the middle panel.

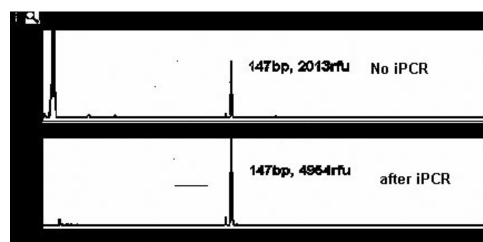

Figure 3. Polymerase chain reaction amplified products after isolation of PCR products (IPCRp).

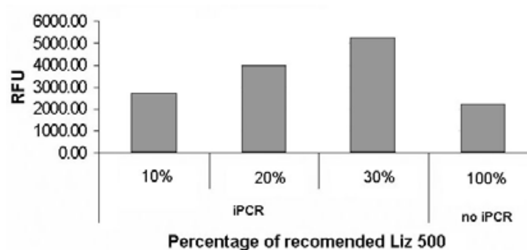

Figure 4. Peak heights of GeneScan™ LIZ 500™ Size Standard with isolation of PCR products (IPCRp) and no IPCRp performed. RFU – relative fluorescent units

the high affinity binding of biotin to streptavidin (8,9). The PCR mix consists of primers, polymerase, buffer, dNTPs, etc. The forward primer is dye-labeled at a 5′-end and the reverse primer is labeled with biotin at a 5′-end. After the PCR, the intended amplification target is in double-stranded configuration, dye-labeled on one strand, and biotin-labeled on the other. Also, there are remains of the enzyme, dNTPs, and unincorporated primers in the PCR mix after the PCR reaction.

After the PCR, biotin-labeled double-stranded product is captured to the streptavidin-coated surface (10,11). Low stringency sodium chloride-sodium citrate (SSC) buffer and 1.5 μL of PCR product is added in each capturing well followed by shaking for 20 minutes. Washing of unincorporated primers and other artifacts follows the capturing phase. Each well is washed three times with 200 μL of SSC. After removal of all liquid from the wells the dye-labeled strand is released with 12 μL of formamide. This yields a highly concentrated dye-labeled, single-stranded DNA target ready to be loaded to a capillary instrument. The procedure does not require any special laboratory equipment and can be accomplished in less than 20 minutes.

Results

Electropherograms of the single-plex (one primer pair) PCR reaction before and after the performance of IPCRp procedure are shown in Figure 2. The unincorporated primers were efficiently washed away. The signal strength was increased from 800 relative fluorescence units (RFU, a measure of quantity of amplified target) to over 3,000 RFU. Electropherograms of the PCR reaction before and after the performance of IPCRp procedure are shown in Figure 3. The peak height was increased from 2,013 RFU with no IPCRp to 4,954 RFU after IPCRp.

The increased sensitivity of detection after the IPCRp is also evident in Figure 4, where

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same peak heights (Y-axes) were obtained with recommended quantity of GeneScan™-LIZ 500™ Size Standard (Applied Biosystems, Foster City, CA, USA.) if only 10% of the recommended quantity were used with IPCRp (X-axes). Only 30% of recommended quantity more than doubled the peak heights for IPCRp product (2,000 for PCR vs. 5,000 RFU for IPCRp).

Discussion

Previously, the streptavidin-biotin DNA capturing was accomplished by a biotynilated probes that were complementary to the target (12). In the IPCRp, the reverse primer that is extended during the PCR serves as a biotynilated probe, thus maximizing the efficiency of capturing and reducing the number of steps. Another big advantage of the IPCRp over similar procedures is that the needed information is the most efficiently transferred from amplified targets to their detection on capillary electrophoresis instruments, thus not requiring, often cumbersome, intermediary hybridization step. The result of the IPCRp method is highly concentrated dye-labeled DNA target that is ready to be loaded on the genotyping platform. The outcome is increase in signal strength by 2 to 5 fold. Such a big increase in signal detection is possible because the sample that is loaded on the detection platform after the PCR contains fewer dye labeled targets than the product of the IPCRp that is virtually 100% dye labeled intended target. Thus, the procedure can be effective in improving the detection of low-copy number DNA.

The IPCRp is an efficient method for concentrating the amplified target DNA, which can enhance the detection.

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