Degenerate Oligonucleotide-Primed PCR (DOP-PCR): Evaluation of its Reliability for Screening of Genetic Alterations in Neoplasia

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Montserrat Sanchez-Cespedes, Paul Cairns, Jin Jen and David Sidransky
Johns Hopkins University School of Medicine, Baltimore, MD, USA

Genetic alterations underlie the development and progression of neoplasia. Screening for genetic alterations in biopsies and premalignant lesions, where the neoplastic tissue analyzed usually constitutes only a small fraction of the cells in the specimen, often is difficult and tedious. Even after successful isolation of DNA, limited quantities allow only a few genetic analyses, limiting the identification of genetic changes in small lesions and the establishment of molecular cancer progression models.

One way to overcome the low DNA abundance problem is to use universal or whole genomic amplification (10). One of the most promising approaches reported for universal genomic amplification is the degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) assay (2). This technique allows the amplification of small genomic DNA samples producing severalfold more DNA for genetic analysis (8). DOP-PCR has been successfully used in cytogenetic analyses, including chromosome painting (6) and comparative genomic hybridization (CGH) (9). However, few data have been reported on the use of DOP-PCR in loss of heterozygosity (LOH) and mutation detection. The purpose of our work was to evaluate the reliability of the DOP-PCR method to identify LOH and gene mutations in primary tumor samples.

Normal lymphocyte and corresponding tumor DNA from two lung tumors displaying LOH for at least one of the microsatellite markers studied were used for DOP-PCR genomic amplification and subsequent microsatellite analysis. Normal lymphocyte and corresponding tumor DNA from one bladder cancer patient (with a mutation in the *PTEN* tumor-suppressor gene) and two head and neck tumors (normal and mutated, respectively, for exon 8 of *p53*) were subjected to DOP-PCR amplification and subsequent PCR and sequencing analysis. Genomic DNA was prepared using standard procedures and quantified spectrophotometrically (7). Serial dilutions of DNA were prepared and used as a template for universal genome amplification. DOP-PCR amplification was performed as previously described (2) using 2 µM of DOP primer (5′-CCGACTGAGNNNNNNATGTGG-3′), 200 µM dNTPs, 0.1% Triton® X-100, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$ and AmpliTaq® DNA Polymerase (Perkin-Elmer, Norwalk, CT, USA) under mineral oil in a

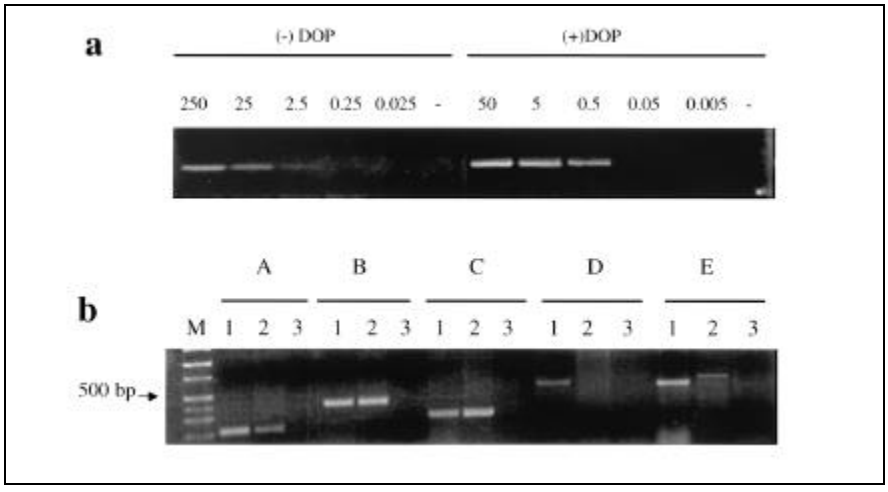

Figure 1. Enrichment and efficiency of DOP-PCR products in PCRs. (a) Efficiency of PCR with different concentrations of genomic DNA with and without DOP-PCR. Amplification product of exon 2 of *PTEN* gene (185 bp) were separated on 1% agarose gel and visualized by ethidium bromide staining The starting DNA concentrations (ng) are indicated at the top. [-], negative control. No PCR product was observed when 0.5 ng of genomic DNA were used in the PCR [(-)DOP]. A strong product band was obtained using 0.5 ng of input DNA after DOP-PCR [(+) DOP]. (b) Size limitation of PCR products. Lane M, HiLo™ DNA Marker (Minnesota Molecular, Minneapolis, MN, USA). (A–D) PCR amplification of exons 2, 5, 7 and 8 of the *PTEN* gene. A, exon 2 (185 bp); B, exon 5 (380 bp); C, exon 7 (263 bp); D, exon 8 (558 bp) and E, PCR amplification of exon 2 (540 bp) of the *p16/MTS1* gene. Lane 1, PCR product from 100 ng of genomic DNA without DOP amplification; lane 2, 2.5 ng of genomic DNA subjected to DOP amplification and lane 3, negative control for each PCR. The band that appears in lane E2 is due to a nonspecific amplification, confirmed by sequence analysis.

50-µL volume. After an initial denaturation of 8 min at 96°C, 2.5 U *Taq* DNA polymerase were added. Thermal cycling conditions were as follows: 8 cycles of 93°C for 1 min, 30°C for 3 min and 72°C for 3 min and then 28 cycles of 93°C for 1 min, 60°C for 1 min and 72°C for 3 min. Negative controls were included to exclude any contamination during each amplification series.

For microsatellite PCR, one primer was labeled with T4 Polynucleotide Kinase (New England Biolabs, Beverly, MA, USA) and [γ-^{32}P]ATP. Serial dilutions of DOP-PCR products and genomic DNA were subjected to 35 cycles of PCR amplification with a 55°C annealing temperature in 10 µL reaction volume. For mutation analysis, 5 µL of each DOP-PCR product were used to amplify exon 8 of *p53* (4), exon 7 of *PTEN* (1) and exon 2 of the *p16* tumor-suppressor gene (5). Cycle sequencing reactions were performed according to the manufacturer's instructions (Perkin-Elmer). Microsatellite and sequencing products were separated by electrophoresis in denaturing 8 M urea 6% polyacrylamide-formamide gels followed by autoradiography.

Our first experiment was designed to test whether DOP-PCR products could be used to analyze LOH by using microsatellite markers. We compared the patterns of allelic loss at markers D3S1289, IFNA and D9S171 in normal and tumor tissue. From 50 to 0.15 ng of genomic DNA were used as a template for the DOP amplification, and 3 µL of the DOP product were used for subsequent microsatellite PCR. We found that DNA quantities as low as 0.15 ng led to robust amplification in the DOP-PCR. However, frequent allelic imbalances, detected as varying band intensities, were observed in the microsatellite analysis when the DOP-PCR product was used as a template. These changes in band intensities were not present in either the normal or tumor tissues before universal genome amplification, suggesting that random skewing of alleles in minute DNA samples used for DOP-PCR can lead to artifacts that appear as LOH. Although we have detected these allele imbalances in all the dilutions

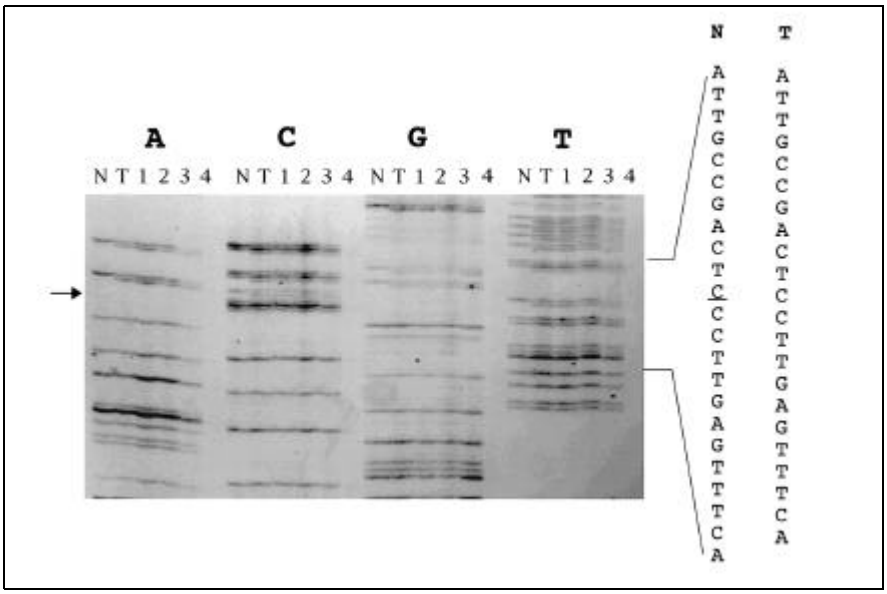

Figure 2. Sequencing gel showing the reproducibility of mutations in DNA templates subjected to universal genomic amplification. N and T, normal and tumor DNA from the same individual. Lanes 1, 2 and 3, sequence of tumor DNA subjected to DOP-PCR. The tumor DNA concentration for DOP amplification was 50, 2.5 and 0.5 ng for lanes 1, 2 and 3, respectively. Lane 4, 0.5 ng of starting tumor DNA without DOP-PCR. The DOP-PCR dilutions show the same 1-bp deletion in codon 244 of exon 7 of the *PTEN* gene (lanes 1–3) compared with the original tumor sample (lane T). The normal tissue (lane N) shows the wild-type sequence, indicating that the deletion is a somatic mutation (not a polymorphism). The arrow on the left indicates the position of the deletion. From the deletion to the 3′ end of the PCR product, each base presents an additional band below (1 bp) according to the pattern expected for a frameshift mutation. On the right it is indicated the normal (N) and the tumor (T) sequence. The base pair deletion is underlined in the normal sequence.

tested, genomic DNA concentrations lower than 5 ng seem to be more likely to show preferential allelic amplification. This observation has been previously reported (3) and indicates that DOP-PCR is not accurate enough to be used as a whole genomic amplification for LOH analysis.

We also studied whether DOP-PCR products were reliable as templates for PCR followed by subsequent sequence analysis. Tumor DNA harboring mutations in either the *p53*, *PTEN* or *p16/MTS1* tumor-suppressor genes were amplified by DOP-PCR. Figure 1a shows a titration experiment indicating the DOP-PCR efficiency. As shown in the ethidium bromide-stained agarose gel, starting DNA of 50, 5 and 0.5 ng in the DOP-PCR allowed the generation of stronger PCR products compared with similar starting DNA concentrations not subjected to DOP amplification. By serial dilutions of the universal amplified products, we have estimated that the DOP-PCR increases from 10 to 100 times the possible number of PCR amplifications for a single locus when 50 ng of genomic DNA are used as a template and up to 10 times when the starting amount of genomic DNA is 0.5 ng. Because the DOP amplification generally generates products below 4 kb (6), we assayed the efficiency of the PCR to amplify different PCR products from 100–600 bp. We observed that most fragments up to 500 bp in size were successfully amplified (Figure 1b). In our hands, we also found that when DOP-PCR was performed with a starting genomic DNA amount below 0.5 ng compared with when higher DNA concentrations were used, the likelihood of successful amplification of a given DNA fragment was lower. Therefore, starting DNA concentrations higher than 0.5 ng and PCR fragments under 500 bp were chosen to evaluate the reliability and efficiency of cycle sequencing on DOP-PCR products. Sequence analysis revealed the reproducibility of DOP-PCR products for general DNA sequencing and, in those samples carrying mutations in the *p53* or *PTEN* genes, the same mutations were clearly detected before and after DOP-PCR using much less genomic DNA. Figure 2 shows a single base deletion in codon 244 of the *PTEN* gene in a bladder tumor DNA, which was also detected in the corresponding DOP-PCR products.

In conclusion, our work indicates that DOP-PCR products from good-quality starting genomic DNA can be used as a template to generate PCR fragments smaller than 500 bp. Although we found that microsatellite analysis is not feasible due to preferential (apparently random) amplification, other PCR-based genetic analyses can be successfully carried out. With this approach, minute quantities of neoplastic DNA can be used to reliably search for gene mutations by cycle sequencing.

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Address correspondence to:

Dr. David Sidransky
Department of Otolaryngology-Head and Neck Cancer Research
Johns Hopkins University School of Medicine
818 Ross Research Building
720 Rutland Avenue
Baltimore, MD 21205-2196, USA
Internet: dsidrans@welchlink.welch.jhu.edu