probe. Retention of the probe on the dialysis bag using the electrophoretic protocol and inefficient binding of the probe to glass beads (as seen with dsDNA below 0.5 kb in size) contribute to probe losses. Since the ss probe does not need to be purified from the LMP agarose gel, loss of the probe associated with the two established protocols described here (particularly with the glass bead protocol) is not a problem. The high yield and ease of recovery of the ss probe not only minimizes the number of steps in the S1 nuclease analysis, but also leads to a reduced-exposure to radioactivity, since less radiolabeled ss product needs to be synthesized initially. Lastly, the high resolving capacity of NuSieve LMP agarose facilitates the preparation of a broad size range of ssDNA probes that do not need further concentration.

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DNA Fingerprinting of Mammalian Cell Lines Using Nonradioactive Arbitrarily Primed PCR (AP-PCR)

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Cross-contamination of eukaryotic cell lines with rapidly growing cells is a particular problem in laboratories working with established cell lines. Isoenzyme analysis, karyotypic characterization and DNA fingerprinting were introduced for quality assurance. In recent years polymerase chain reaction (PCR)-based fingerprint techniques have been used as highly specific and rapid supplemental tools for characterization of eukaryotic cell lines. Previously, it was shown that simple and reproducible fingerprints of complex genomes can be generated using single arbitrarily chosen primers and the PCR (6). However, most reports of the arbitrarily primed PCR (AP-PCR) used radioactive detection of the fingerprints, which may hamper its applicability in routine control of cell cultures. Radioactive techniques require expensive laboratory equipment, and the techniques are time-consuming. Moreover, these techniques are hazardous to laboratory personnel and the environment. Nonradioactive PCR-based DNA-fingerprinting techniques, however, are not as sensitive as radioactive methods. Here we demonstrate the use of silver staining of AP-PCR fingerprints in order to identify species-specific profiles.

Genomic DNA was prepared from cell lines and tissues of different species by standard protocols. Chinese hamster ovary (CHO) cells, NIH 3T3 and NIH 3T6 mouse fibroblasts were obtained from ATCC (Rockville, MD, USA); gerbil cells were established from gerbil fibroblasts; rat neurinoma cell line RN-4.41 and rat glioma cell line RGL.3 were established from experimentally induced rat tumors (5); human cell lines RT4, J82 and HU609 were established from human urothelial carcinomas (3); and Mw10 was from human meningioma. In addition, one human glioma cell line (Gw51) was used in which we performed a cross-contamination with cells from the established rat glioma cell line RGL.3. For this purpose, one hundred RGL.3 cells were mixed with 10^6 slowly growing human glial cells. DNA from this contaminated cell line was isolated after 5 and 10 passages. During this time the cell line showed dramatic changes in growth characteristics.

Three different arbitrarily chosen primers (JS1: 5'-GAT AGC CAG CAC AAA GAG AGC TAA -3'; JS2: 5'-CGA CCG TGT TTT GCA AAC AGA TGT -3' and ZF3: 5'-CCC CAC CGG AGA GAA ACC -3') were used for AP-PCR (4). PCR amplifications were performed at two concentrations of template DNA with 50 ng and 200 ng purified genomic DNA in a reaction mixture containing 1.5 µM each primer, 0.2 mM each dNTP, 50 mM KCl, 10 mM Tris-HCl, pH 8.8, at 25°C, 4 mM MgCl2, 1 mM dithiothreitol (DTT), 1 U Tag DNA Polymerase (Promega, Madison, WI, USA) in a final volume of 50 µL. The PCR was carried out in a MJ Research Thermocycler (Watertown, MA, USA) for 4

Figure 1. AP-PCR fingerprints of different cell lines using primer JS2. Cell lines are (from right to left) gerbil, Chinese hamster (CHO), mouse (3T6, 3T3), human (J82, RT4, HU609, Mw10, Gw51) and rat (RGL.3, RN-4.41). Cont. 0, 5 and 10 indicate contamination experiment of Gw51 with rat glioma cell line RGL.3 immediately after contamination (0), and after 5 and 10 passages. Individual polymorphisms in mouse fibroblast cell lines 3T3 and 3T6 are indicated using arrowheads.

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cycles at low stringency (94°C for 1 min; 40°C–45°C, depending on primer, for 5 min; 72°C for 5 min) and 30 cycles of amplification at high stringency (94°C for 1 min; 60°C for 1 min; 72°C for 2 min; last cycle 72°C for 10 min). Two and a half microliters of each reaction were added to 5 of formaldehyde-dye mixture and subsequently run on 6.7% polyacrylamide/7 M urea gels (3 h, 2000 V, 50°C) in a Sequi-Gen® sequencing gel chamber (Bio-Rad, Hercules, CA, USA). Silver staining was performed according to the method of Budowle et al. (2) in a modified staining container (1) of the Sequi-Gen gel chamber. Briefly, the gels were placed in 10% ethanol (5 min), oxidized in 1% nitric acid (3 min), placed in 0.012 M silver nitrate (20 min) and reduced in a solution containing 0.28 M sodium carbonate and 0.019% formalin until bands developed. Reduction was stopped with 10% glacial acetic acid (2 min), and the gels were placed in distilled water (2 min). Subsequently, the gels were dried in vacuo and stored for permanent record.

Using AP-PCR, we generated species-specific and reproducible DNA fingerprints, which were highly polymorphic between different species (Figure 1). In addition, individual polymorphisms were found in the background of species-specific patterns. The mouse and human cell lines and tissues showed diagnostic polymorphisms, which allowed identification of different individuals. However, the number of inter-individual polymorphisms was only 3–5 in a background of 30–50 bands (10%) generated by distinct primers. In contrast, rat cell lines were from inbred strain BDIX, and no differences were found in fingerprints generated from these sources. These results were reproducible under constant conditions; i.e., we found identical band patterns in repeated (up to 5) experiments using the same DNA and primer. However, the quantity of template DNA was crucial to the generation of reproducible profiles; differences in band patterns could be observed when DNA amounts were below 10 ng or above 2 µg. Varying DNA concentrations between 50 ng and 1 µg gave identical DNA fingerprints. To estimate the percentage of cells detectable by AP-PCR in the background of another species, we performed an experiment using a defined mixture of human and rat DNAs (Figure 2). Using this approach, we observed species-specific PCR fragments at the level of at least 0.1%. Under normal cell culture conditions of five passages after contamination with rapidly growing rat cells, no human fingerprints were distinguishable, indicating that the amount of human cells was less than 0.1%. The identification of contamination with cells of the same species is more difficult and depends on the number of polymorphic fragments in species-specific profiles.

Using AP-PCR we were able to generate species-specific and individual diagnostic DNA fingerprints. Thus, the method is reliable for identification of cross-contaminations of cell lines at the level of at least 0.1% contaminating cells. AP-PCR amplification depends only on the use of one primer and subsequent gel electrophoresis. The major advantage of polyacrylamide gel electrophoresis, compared to agarose gel electrophoresis, is the distinction of many bands at high resolution. Unlike previous AP-PCR approaches, our method does not require radioactive labeling and detection, making it an easy-to-use and rapid technique.

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