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Brief Communication

Species Differentiation of DNA using Dot-Blots with Biotinylated or ^{32}P-Labeled Genomic DNA Probe

Recombinant DNA technologies offer new methods of detecting animal diversity at various levels. DNA »fingerprinting« using cloned repetitive sequences can be used to distinguish between individuals within breeds and may thus be used for paternity testing (*Jeffreys et al.* 1985). The forensic value of evidence of this kind has given guarded approval by the UK Immigration Advisory Service in its annual report (*Nature* 1987). At the between-species level labeled total genomic DNA was used by *Durnam et al.* (1985) as probe in an in situ hybridization for speciesverification of chromosomal materials in human-rodent hybrid cell lines, and we recently described the use of ^{3}H-labeled genomic pig DNA probe for detection af swine chromosomes in swine-hamster hybrid cell lines (*Thomsen & Christensen* 1986).

Discrimination between related DNA sequences by dot-blots was described by *Kafatos et al.* (1979) for estimating evolutionary changes. Based on this work we here describe the verification of the species of origin for 2 cell lines taking advantage of recent development of highly sensitive non-autoradiographic dot-blots (*Chan et al.* 1985).

The procedure involves isolation of genomic DNA of the material of interest and preparation of probe from individuals of the relevant species. So far, we have extended the range of available probes to include cattle, swine, dog, cat, human, mink and chicken. Probe was prepared by isolating genomic DNA as previously described (*Thomsen & Christensen* 1986) and either labeled with ^{32}P or biotinylated, in both cases using a conventional nick-translation reaction (*Maniatis et al.* 1982). DNA to be tested was isolated by the same protocol, denatured by heating at 95°C for 4 min and chilled on ice for 2 min. One microgram of sample was spotted onto a Hybond N membrane (Amersham International) in 2 µl aliquots and the membrane was then wetted in 1.5 mol/l NaCl, 0.5 md/l NaOH for 1 min followed by 1 min in 1.5 md/l NaCl, 0.5 md/l Tris-HCl (pH 7.2) 0.001 md/l Na_2EDTA. After air drying it was irradiated with UV-light (254 nm) for 5 min.

Hybridization of membrane-bound DNA with labeled probe was initiated by incubating membrane at 65°C in prehybridization solution as described by *Maniatis et al.* (1982) for 1 h followed by hybridization for 16-18 h at 65°C after addition of probe to the prehybridization solution to give a final concentration of 10-20 ng probe/ml hybridization solution. Post-hybridization washes were performed by incubating the membrane in 2 changes of 50 ml 2 × SSC (1 × SSC = 0.5 md/l NaCl, 0.015 md/l Na-citrat, pH 7.0) at 65° C for 15 min in 50 ml 2 × SSC, 0.1% SDS for 30 min and in 50 ml 0.1 × SSC for 10 min, all steps at 65°C.

Macroscopic autoradiography was performed for the ^{32}P-labeled probe, whereas the

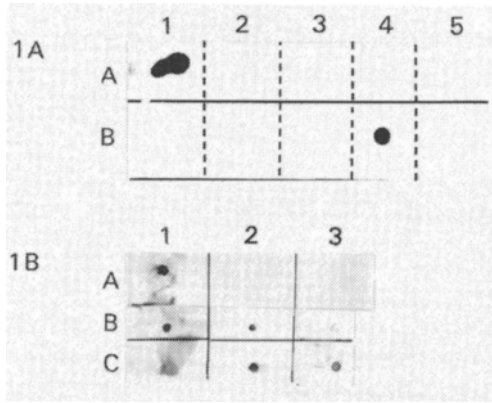

Figure 1A. Verification of swine origin of cell line PD 5 using ^{32}P labeled swine probe. Row A = sample DNA: A1) Porcine kidney cell line PD 5. A2) Bovine kidney cell line NBK. A3) and A4) are unknown DNA samples delivered as internal control. Row B = standard DNA: B1) Hamster cell line B14-150. B2) Mouse cell line P3/X 63 - Ag 8 U 1 (PU). B3) Human lymphocyte. B4) Porcine lymphocyte. B5) Feline lymphocyte.

Figure 1B. Species-determination of cell line NBK and estimation of dot blot sensitivity using biotinylated bovine genomic probe. Row A = standard DNA: A1) 3 ng biotinylated bovine genomic DNA. A2) 1 ug porcine lymphocyte. A3) 1 ug human lymphocyte. Row B = bovine lymphocyte DNA: B1) 50 ng. B2) 10 ng. B3) 1 ng. Row C: Bovine kidney cell line DNA: C1) 50 ng. C2) 10ng. C3) 1 ng. The missing reaction in C1 is due to an air bubble.

biotinylated DNA was detected by the Bluegene system (BRL). We were thus able to choose a discrimination level in the conditions used that allowed positive demonstration of the species used as probe, leaving negative controls essentially blank.

Figure 1 A demonstrates the swine origin of the cell line PD 5 (Duphar, Holland). It also demonstrates absence of significant signal of negative controls and a significant signal for the swine DNA included as a positive control. Figure 1 B demonstrates the bovine origin of cell line NBK (Duphar, Holland) as well as the positive identification of 1 ng test DNA using biotinylated probe. Further experiments (data not shown) established this to be the sensitivity level for both ^{32}P-labeled and biotinylated probes under the assay conditions used. By optimizing conditions the sensitivity may nevertheless be increased, presumably 10-fold. For example the use of cloned species specific DNA sequences as probe might increase the signal: noise ratio.

The technique has various potential uses as a diagnostic tool. The present example concerned a commercial cell line, for which the species identification was essential for registration of a new cell-line-based vaccine. The method will be used for further tests of this kind, but may also be useful to other fields of laboratory diagnosis. For food quality control and for forensic medicine a determination or verification of the species of a sample is sometimes wanted and a test method based on the described principle might in some instances be useful.

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