

STREAMLINING VNTR ANALYSIS. A FAST PROCEDURE FOR NON ISOTOPIC DNA PROFILING.

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Introduction.

Several modifications to the general procedure used for RFLP analysis of VNTR loci have been developed through the years. These modifications include the use of simple protocols for DNA isolation, fast capillary transfer of DNA, fast hybridization procedures and the use of non-isotopic detection. We have selected or developed a combination of methods that have resulted in a significant increase in speed and efficiency of VNTR based DNA profiling. The protocol described below allows the laboratory to obtain consistent results within a period of 2 days or less.

Materials and Methods.

DNA isolation. DNA was prepared from blood samples by Proteinase K treatment and selective precipitations (non-organic procedure) as described by Grimberg et al., or using guanidinium salts and chloroform (non-phenol extraction method) by a modification of the method of Chomczynski and Sacchi (2). The general process consisted of: washing the blood cells (0.5 to 1 ml of blood) with buffer containing Triton X-100 and collecting nuclei by centrifugation.

In the non-organic procedure, nuclei were lysed by treatment with Proteinase K (1). The amount of DNA in each sample was estimated with a yield gel by comparing the fluorescence of the sample against known amounts of DNA. Approximately 3 μg of DNA was digested with 20 fold excess of the restriction enzyme PstI or HaeIII. Following digestion, residual proteins or peptides were removed with LiCl. DNA was precipitated with 2 volumes of ethanol, washed with 70% ethanol and dissolved after drying in 10 μl of H_2O .

In the non-phenol extraction method, nuclei were lysed by addition of guanidinium thiocyanate (2) and extraction with chloroform. DNA was precipitated with a half volume of isopropanol, washed with ethanol and dissolved after drying in 50 μl of H_2O .

DNA quantitation. The amount of DNA in each sample was estimated with a yield gel. Approximately 3 μg of DNA was digested with restriction enzyme. DNA (0.5 to 1 μg) was fractionated by electrophoresis in trays (12 x 21.5 cm or 12 x 28 cm) containing a 2 mm thick, 1.0% or 0.8% IDna agarose gel (FMC Bioproducts) casted directly on to a nylon membrane.

Fractionation of DNA. Electrophoresis was done in an horizontal apparatus for 16 to 18 hrs. After electrophoresis, the membrane/gel combination was soaked (15 min) in an alkaline solution to denature the DNA.

DNA transfer. DNA was transferred to a nylon membrane (Pall Biotodyne B or MSI Magna) by capillary transfer (30 min). The nylon membranes were dried in an oven at 80 °C for 1 hr or for 2 to 3 min in a microwave oven.

DNA hybridization. Hybridization (15 to 20 min) was done using alkaline phosphatase labeled probes (Lifecodes Corp). Membranes were washed and incubated with Lumi-Phos™ 480 (Lumigen, Inc) as substrate. The light emission was detected with X-ray film by exposing the membranes for 2 to 3 hrs at 37 °C or overnight at room temperature.

Results and Discussion.

The process of DNA typing VNTR loci can be divided into the following four major sections: isolation and digestion of DNA, fractionation by electrophoresis, transfer to membrane and detection of alleles. The procedure routinely used in our laboratory for the isolation and digestion of DNA has been described by Grimberg et al. (1). We have also examined the use of a chaotropic agent, such as guanidinium thiocyanate, for DNA isolation. Both procedures produce DNA suitable for digestion with restriction enzyme. The method using the chaotropic agent required less time to complete than the Proteinase K method. However, the DNA recovered was not as pure as with Proteinase K and the DNA fragments migrated somewhat slower than highly purified DNA indicating that the procedure may need additional changes. Fractionation of DNA fragments was done overnight (16 to 18 hrs). DNA from the thin gels were transferred to the nylon membrane over which the gel had been casted. Both neutral and positively charged membranes have been used successfully. The only difference in the procedure was that for the amphoteric membrane the gel was soaked in neutralizing solution prior to transfer, while for the charged membrane the transfer was done with alkaline solution. A comparison of various transfer times showed transfer was complete in approximately 30 minutes. Using 6 mm thick gels the equivalent transfer procedure required 3 hours. After transfer the membrane was quickly dried for a 2 to 3 minutes in a microwave oven. The non-isotopic method used for the detection of the polymorphic loci was based on the use of chemiluminescence. The initial procedure developed in our lab (3,4) has been further simplified and the quality of the results improved by the elimination of several steps and changes in formulation. A flow chart summarizing the steps used in this process and the duration of each step is summarized in Table 1.

Table 1. Procedure for DNA profiling with VNTR loci.

Day 1.	Time (hr)
1. DNA Isolation and digestion with restriction enzyme.	
Cell Lysis	0.5
Nuclear lysis	2.0
DNA quantitation (yield gel)	1.0
Restriction enzyme digestion	2.0
Salting out	0.25
DNA precipitation	0.5
Sub-total:	6.25

2. DNA fractionation.

Gel electrophoresis	16.0
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Day 2.

3. DNA transfer.

Denaturation	0.25	
Southern Transfer	0.5	
Drying	0.1	
Sub-total:	0.85	

4. DNA hybridization to AP-probes.

Hybridization	0.25	
Washing	1.0	
Exposure at 37 °C	3.0	
Sub-total:	4.25	

TOTAL:	~ 28 hrs
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The main objective of this manuscript was to summarize our efforts to shorten and simplify the procedures required to produce DNA profiles for paternity cases using VNTR loci. The procedure outlined above uses several simple changes to the general methods used for RFLP analysis that shortens the time required to complete routine paternity cases to less than 2 days.

References.

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